Research Article

Postharvest UV-B exposure drives changes in primary metabolism, phenolic concentration, and volatilome profile in berries of different grape (*Vitis vinifera* L.) varieties

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Abstract

BACKGROUND: The ultraviolet-B (UV-B) radiation can alter grape metabolism during berry development, but little is known on the effect of postharvest UV-B exposure. In this study, we evaluated the effect of postharvest UV-B exposure on berry primary and secondary metabolites in four grapevine varieties (Aleatico, Moscato bianco, Sangiovese, and Vermentino) in order to evaluate the possibility to increase the grape quality and its nutraceutical properties.

RESULTS: The treatment did not significantly affect the berry primary metabolism in terms of organic acids, carbohydrates, and amino acids profile, regardless of the variety. UV-B exposure reduced the total anthocyanin content, particularly the tri-substituted and di-substituted forms in Aleatico and Sangiovese, respectively. An overall negative effect of UV-B irradiation on the flavonols profile of Aleatico, Moscato bianco, and Vermentino berries was found, whereas it enhanced the quercetin, myricetin and kaempferol concentration in Sangiovese. The free fraction of berry volatile organic compounds increased in UV-B-treated Aleatico and Moscato bianco berries, especially C_{13}-norisoprenoids and volatile phenols, as well as key monoterpenes, such as the linalool derivatives. However, higher concentrations of glycosylated monoterpenes and C_{13}-norisoprenoids were measured in Sangiovese and Vermentino berries treated with UV-B.

CONCLUSION: This study provides new insights on the effect of postharvest UV-B radiation on berry secondary metabolism, highlighting a different modulation between varieties and suggesting the potential use of this technique to increase some nutraceutical and quality characteristics of grape berry.
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INTRODUCTION

Sunlight is one of the most important climatic factors influencing grape composition.[1-3] Light exposure, in terms of quality and quantity strongly impacts the biosynthesis and accumulation of berry secondary metabolites, which are the leading factors determining berry and wine quality. A wide literature reported that different grape varieties exposed to sunlight radiation increased the level of phenolic compounds.[4-6] In particular, flavonols are among the most light-responsive phenolic subclasses, strongly correlated with sunlight exposure especially after veraison.[7-9] Higher anthocyanin content was also detected in berries exposed to high-light conditions due to the up-regulation of many relative biosynthetic genes.[10-14] The concentration of berry aroma compounds was

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affected by radiation level as well. Terpenes are sensitive to light and increasing the exposure to sunlight radiation enhanced their concentration in different grape varieties.[15,16] Similarly, C_{13}-norisporenoids derived from photoprotective pigments such as carotenoids, are highly related to the level of sun exposition during berry development.[17,18]

Vines grown in the vineyard are also normally exposed to ultraviolet-A (UV-A, 315–400 nm) and ultraviolet-B (UV-B, 280–315 nm) radiations which are a significant component of solar radiation reaching the heart's surface. Since vines are generally adapted to high UV doses in the environment, it does not represent a stress factor,[8] but rather an environmental signal that modulates vine physiological and biochemical traits by inducing an accumulation of secondary metabolites.[19,20] Generally, UV-B radiation can trigger the biosynthesis of many phenolic subclasses, e.g., anthocyanins, flavonols, and tannins through an overexpression of the related biosynthetic genes.[21,22]

Field experiments investigating the effect of UV-B exposure on grapevines, showed an accumulation of several phenolics compounds and subclasses, e.g., anthocyanins, as an acclimation response towards UV-B radiation.[23-25] UV-B radiation also stimulates the biosynthesis of several volatile organic compounds (VOCs), especially monoterpenes, mainly thanks to their protective role in response to many abiotic and biotic stresses.[26-28]

However, studies on the UV-B effects on grape berries after harvest are scant. Sheng *et al.*[29] reported that table grapes irradiated with UV-B (3.6 kJ m^{-2}) up to 28 days after harvest at 4 °C resulted in the increase of total phenolics, flavonols, and anthocyanins, although no effects were observed for specific phenolic compounds, e.g., gallic and caffeic acids. In particular, the UV-B-driven accumulation of phenolics is mainly due to the overexpression of the phenylpropanoid biosynthetic genes, such as the phenylalanine ammonia lyase (PAL), the chalcone synthase (CHS), the flavanone 3-hydroxylase (F3H), and the anthocyanidin synthase (ANS).[21,29,30] When considering the postharvest, a positive correlation between UV-B treatment and phenolic accumulation has also been reported in many other fruit species, such as tomato,[31,32] apple[33,34] and peach.[35-38]

To date, few studies investigated the postharvest UV-B-driven changes in the secondary metabolism of non-climacteric fruits, especially grape berries.[29,39] In the light of this, the present research aimed to investigate the UV-B-induced modification during postharvest on berry primary and secondary metabolites, particularly flavonols and anthocyanins, as well as on the berry aroma profile, in four wine grape varieties, Aleatico, Moscato bianco, Sangiovese, and Vermentino.

MATERIALS AND METHODS

Plant material, berry samples and UV-B treatment
The study was conducted on berries of four varieties important for Tuscany viticulture and described in the Italian Vitis Database (https://vitisdb.it/). The berries were harvested from eight-year-old grapevines (*Vitis vinifera* L.) varieties Aleatico (ALE), Moscato bianco (MOS), Sangiovese (SAN) and Vermentino (VER) grafted on 110R and grown at the experimental farm of the experimental farm of the Department of Agriculture Food and Environment of the University of Pisa, Italy (43.732153 N; 10.465836 E). Vines were grown outdoor in 50 L container (40% peat and 60% silty-loam soil), at 4.2 m × 0.9 m distances in rows north–south oriented. All vines were trained according to the Guyot system leaving one spur with two count buds and one cane with 56 count

buds.[40,41] Fertilizers were supplied via fertigation in several batches between bud break and leaf fall.[9]

The berries were harvested at different total soluble solids (TSS) levels according to the most appropriate content for winemaking in the area. Six replicates of 180 berries were randomly collected from five vines each for ALE and MOS (30 vines per variety) and six replicates of 230 berries from five vines each for SAN and VER (30 vines per variety). The berries were picked from different bunches without removing the pedicels, and carefully selected to be uniform in color and dimension, unwounded, undamaged and without visual defects. Three replicates per variety were subjected to the UV-B treatment whereas the other three replicates were untreated and used as control.

The berry fresh weight (FW) was measured within 1 h from harvest and after UV-B treatment. The TSS, pH and titratable acidity (TA) were determined before UV-B exposure on an aliquot of 100 berries per replicates. The berry juice TSS was measured with a digital refractometer (DBRwine, HM Digital Ltd., Seoul, Korea), and pH determined with a pH meter (Hanna Instruments, Woonsocket, RI, USA). A 10 mL aliquot of juice was titrated with 0.1 N sodium hydroxide (NaOH) to an endpoint pH of 8.2 to determine TA, expressed as g L^{-1} of tartaric acid.

The remaining aliquot of berries of three replicates per variety, was subjected to the UV-B treatment, which was conducted at 22 ± 0.1 °C and $85 \pm 5\%$ relative humidity (RH) in proper climatic chambers supplied with three UV-B tubes (Philips Ultraviolet-B Narrowband, TL 20 W/01 – RS, Koninklijke Philips Electronics, Eindhoven, the Netherlands). The UV-B conditions were chosen based on preliminary UV-B dose–response tests and the berries were placed on a single layer to avoid overlapping. A UV-B irradiance of 1.36 W m^{-2} was provided at fruit height and the treatment lasted 96 h (4 days), corresponding to a total UV-B dose of 470.02 kJ m^{-2}.

The remaining aliquot of control berries replicates (not UV-B irradiated) were kept in the dark in a separated climatic chamber at the same temperature and humidity conditions.

At the end of the UV-B treatment, 50 (for ALE and MOS) or 100 (for SAN and VER) berries from both the control and the UV-B-treated replicates were used for the VOCs analysis, while the remaining 30 berries of each replicate, regardless of the grape varieties, were immediately freeze dried for the primary metabolites and the phenolic determination.

Berry primary metabolites determination
The determination of primary metabolites was carried on using nuclear magnetic resonance (NMR) spectroscopy. Freeze-dried berries were grinded to a fine powder with mortar and pestle. Then 100 mg were extracted with 750 μL of methanol-d_4 and 750 μL of potassium dihydrogen phosphate (KH_2PO_4) buffer in deuterium oxide (D_2O) pH 6.0, containing 0.01% 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt (TMSP, Sigma-Aldrich, St Louis, MO, USA). The mixture was vortexed at ambient temperature for 1 min, sonicated for 20 min, and centrifuged at $11500 \times g$ for 5 min. After centrifuging 600 μL of the supernatant was transferred to a 5 mm NMR tube.[42] Proton (^{1}H)-NMR spectra were recorded at 25 °C on a Bruker Advance III HD spectrometer equipped with a Smart Probe™ with *z*-axis gradients (Bruker BioSpin GmbH, Rheinstetten, Germany), operating at the ^{1}H-NMR frequency of 500.18 MHz. The spectrometer transmitter was locked to methanol-d_4 and all the spectra were recorded with the Bruker pulse sequence 'noesypr1d' with presaturation of the water signal at 4.844 ppm. Each sample was collected into 64k data points after 128 scans and four dummy

scans using a spectral width of 8000 Hz. The receiver gain was set to 90, the relaxation delay of 1 s, the acquisition time of 4 s and mixing time of 0.1 s. The free induction decay was multiplied by 0.3 Hz line broadening before Fourier transformation. TMSP was used for calibration at 0.0 ppm. The ^{1}H-NMR spectra were manually phased and baseline-corrected in Topspin version 3.61. Chenomx NMR suite version 8.5 software, professional edition (Chenomx Inc., Edmonton, Canada) was used for quantification, using an in-house library, and published annotated spectra.[42]

Berry flavonols and anthocyanins determination

Berry flavonol and anthocyanin concentration was determined on three replicates of 30 freeze dried berries per treatment per variety. The analysis was carried out following the procedure described by Downey and Rochfort[43] and slightly modified as reported by Palai et al.[9] An aliquot of 0.4 g of freeze-dried berries was extracted with 2 mL of pure methanol for 30 min on an orbital shaker. After centrifugation (10 000 × g, 15 min), the supernatant was filtered with a nylon filter (0.22 μm) and injected into an Agilent high-performance liquid chromatography (HPLC) 1260 infinity system with a diode array detector (DAD) and a Poroshell 120 EC-C18 column (4.6 mm × 150 mm, 2.7 mm; both from Agilent Technologies, Santa Clara, CA, USA). Chromatographic separation was achieved by using 10% formic acid in water as eluent A and 10% formic acid in methanol as eluent B, following a linear gradient: from 17% to 40% B in 12 min, from 40% to 50% B in 5 min, from 50% to 100% B in 3 min, 100% B for 7 min. The chromatographic column was thermostated at 40 °C and the flow rate was 0.8 mL min⁻¹. Anthocyanins and flavonols were detected at 520 and 354 nm, respectively. Identification of the individual component peaks was performed by making a comparison of retention times and UV-visible absorption data of analytical standards (malvidin-3-glucoside chloride, cyanidin-3-glucoside chloride, petunidin-3-glucoside chloride, peonidin-3-O-glucoside chloride, delphinidin-3-glucoside chloride, quercetin-3-glucoside, kaempferol-3-glucoside, myricetin-3-glucoside) and with those found in the literature.[43] Quantification was performed using the same external analytical standards to build the calibration curves.

Berry VOCs analysis

On three replicates (each replicate consisting of 50 berries from ALE and MOS, and 100 berries from SAN and VER) per treatment were determined free and glycosylated VOCs by solid phase extraction (SPE), following the protocol reported in detail by D'Onofrio et al.[44] Briefly, the skins were separated, extracted with 20 mL of pure methanol, and added with flesh and juice to 150 mL of pH 3.2 tartaric buffer solution (2 g L⁻¹ sodium metabisulphite, 5 g L⁻¹ tartaric acid and 22 mL L⁻¹ NaOH 1 N). After homogenization and centrifugation, the supernatant was inoculated with a pectolytic enzyme (Vinozym FCE G; Novozymes, Bagsværd, Denmark) and incubated over night at room temperature. A total of 200 μL of 1-heptanol (40 μg mL⁻¹) was added to this extract as internal standard and eluted through a 5 g C18 Cartridge (Mega Bond Elut; Agilent Technologies). The free and glycosylated fraction was then separately extracted through the specific SPE protocol.[45] The chromatographic analysis was carried out using an Agilent 7890A gas-chromatograph coupled with an Agilent 5975C quadrupole mass spectrometer (Agilent Technologies). The carrier gas was helium at a constant flow rate of 1 mL min⁻¹ whereas the capillary column was an HP-Innowax [30 m length, 0.25 mm inner diameter (i.d.), 0.25 mm film

Table 1. Harvest date (day of the year, DOY), berry fresh weight (FW) before and after ultraviolet-B (UV-B) treatment, total soluble solids (TSS), pH and titratable acidity (TA) measured in berries of Aleatico (ALE), Moscato bianco (MOS), Sangiovese (SAN) and Vermentino (VER) grapevines (Vitis vinifera L.) subjected to postharvest UV-B treatment

	ALE			MOS			SAN			VER		
	CTR	UV-B	P	CTR	UV-B	P	CTR	UV-B	P	CTR	UV-B	P
Harvest date (DOY)	253	253		251	251		257	257		257	257	
Berry FW pre UV-B (g)	2.08 ± 0.08	2.08 ± 0.14	n.s.	2.72 ± 0.05	2.91 ± 0.15	n.s.	2.18 ± 0.14	2.21 ± 0.23	n.s.	2.93 ± 0.15	2.73 ± 0.36	n.s.
Berry FW post UV-B (g)	2.04 ± 0.12	2.04 ± 0.11	n.s.	2.66 ± 0.05	2.85 ± 0.25	n.s.	2.15 ± 0.28	2.01 ± 0.12	n.s.	2.88 ± 0.05	2.67 ± 0.35	n.s.
TSS (°Brix)	26.80 ± 0.52	26.73 ± 0.23	n.s.	25.66 ± 0.57	26.06 ± 0.11	n.s.	23.60 ± 1.05	23.06 ± 0.23	n.s.	25.20 ± 0.3	25.13 ± 0.9	n.s.
pH	3.42 ± 0.04	3.32 ± 0.10	n.s.	3.45 ± 0.04	3.53 ± 0.03	n.s.	3.12 ± 0.06	3.18 ± 0.09	n.s.	3.31 ± 0.15	3.41 ± 0.02	n.s.
TA (g L⁻¹ tartrate)	7.95 ± 0.12	7.20 ± 0.15	**0.008**	6.05 ± 0.28	5.75 ± 0.22	n.s.	7.60 ± 0.31	7.32 ± 0.18	n.s.	6.02 ± 0.33	5.47 ± 0.49	n.s.

Note: Values are means of three replicates per treatment ± standard deviation. Significant differences were determined by Student's t-test ($P \leq 0.05$) within each variety.
Abbreviation: CTR, control berries (not UV-B treated); UV-B, berries treated with UV-B light; n.s., not significant.

Table 2. Organic acids, amino acids, and carbohydrate profile in berries of Aleatico (ALE), Moscato bianco (MOS), Sangiovese (SAN) and Vermentino (VER) grapevines (*Vitis vinifera* L.) subjected to post-harvest ultraviolet-B (UV-B) treatment

Metabolic class	Compound	ALE CTR	ALE UV-B	ALE P	MOS CTR	MOS UV-B	MOS P	SAN CTR	SAN UV-B	SAN P	VER CTR	VER UV-B	VER P
Acids	Acetic acid	0.02 ± 0.01	0.02 ± 0.01	n.s.	0.03 ± 0.01	0.04 ± 0.01	**0.03**	0.03 ± 0.02	0.03 ± 0.01	n.s.	0.01 ± 0.01	0.01 ± 0.01	n.s.
	Malic acid	2.00 ± 0.30	1.93 ± 0.14	n.s.	2.01 ± 0.30	2.51 ± 0.20	n.s.	2.95 ± 0.30	2.71 ± 0.28	n.s.	3.20 ± 0.42	3.40 ± 0.07	n.s.
	Syringic acid	0.03 ± 0.01	0.02 ± 0.02	n.s.	—	—	—	0.03 ± 0.01	0.03 ± 0.01	n.s.	—	—	—
	Tartaric acid	1.45 ± 0.09	1.45 ± 0.13	n.s.	1.24 ± 0.28	3.58 ± 3.08	n.s.	0.22 ± 0.23	0.07 ± 0.01	n.s.	2.99 ± 1.06	1.24 ± 0.28	n.s.
	TOTAL	3.49 ± 0.21	3.44 ± 0.12	n.s.	3.27 ± 0.33	6.13 ± 1.83	n.s.	3.22 ± 0.09	2.84 ± 0.17	n.s.	6.20 ± 0.84	5.25 ± 0.07	n.s.
Amino acids	Alanine	0.05 ± 0.01	0.05 ± 0.01	n.s.	0.03 ± 0.01	0.04 ± 0.01	n.s.	0.10 ± 0.01	0.10 ± 0.01	n.s.	0.04 ± 0.01	0.04 ± 0.01	n.s.
	Arginine	0.34 ± 0.01	0.30 ± 0.04	n.s.	—	0.12 ± 0.21	n.s.	0.55 ± 0.10	0.58 ± 0.03	n.s.	—	—	—
	Isoleucine	0.03 ± 0.01	0.02 ± 0.01	**0.02**	0.02 ± 0.01	0.02 ± 0.01	n.s.	0.03 ± 0.01	0.02 ± 0.01	**0.05**	0.02 ± 0.01	0.02 ± 0.01	n.s.
	Glutammine	0.12 ± 0.01	0.07 ± 0.06	n.s.	0.02 ± 0.04	0.07 ± 0.03	n.s.	0.10 ± 0.05	0.11 ± 0.06	n.s.	0.05 ± 0.05	0.07 ± 0.01	n.s.
	Proline	0.43 ± 0.01	0.41 ± 0.10	n.s.	—	0.06 ± 0.11	n.s.	1.50 ± 0.21	1.35 ± 0.08	n.s.	0.33 ± 0.04	0.33 ± 0.08	n.s.
	Threonine	0.03 ± 0.01	0.02 ± 0.01	n.s.	0.01 ± 0.01	0.01 ± 0.01	n.s.	0.06 ± 0.01	0.06 ± 0.01	n.s.	0.01 ± 0.01	0.01 ± 0.01	n.s.
	Valine	0.03 ± 0.01	0.02 ± 0.01	n.s.	0.02 ± 0.01	0.02 ± 0.01	n.s.	0.05 ± 0.01	0.04 ± 0.01	n.s.	0.02 ± 0.01	0.02 ± 0.02	n.s.
	TOTAL	1.02 ± 0.02	0.89 ± 0.09	n.s.	0.10 ± 0.02	0.34 ± 0.20	n.s.	2.38 ± 0.19	2.26 ± 0.10	n.s.	0.47 ± 0.05	0.49 ± 0.05	n.s.
Carbohydrates	Fructose	242.4 ± 20.9	253.0 ± 9.8	n.s.	264.9 ± 8.8	298.4 ± 50.6	n.s.	253.2 ± 8.0	246.3 ± 4.7	n.s.	266.5 ± 12.1	263.9 ± 8.8	n.s.
	Glucose	250.7 ± 13.8	266.6 ± 13.9	n.s.	267.5 ± 11.8	277.6 ± 18.7	n.s.	262.0 ± 5.3	252.9 ± 8.7	n.s.	291.2 ± 31.8	278.4 ± 3.9	n.s.
	Sucrose	1.2 ± 0.5	1.0 ± 0.1	n.s.	0.7 ± 0.1	0.7 ± 0.3	n.s.	1.0 ± 0.6	0.9 ± 0.2	n.s.	1.2 ± 0.6	0.6 ± 0.1	n.s.
	TOTAL	494.3 ± 19.5	508.1 ± 1.2	n.s.	533.0 ± 11.2	576.7 ± 40.1	n.s.	516.2 ± 2.9	500.1 ± 7.7	n.s.	558.8 ± 23.2	543.0 ± 6.8	n.s.

Note: Values are means of three replicates per treatment ± standard deviation. Significant differences were determined by Student's *t*-test ($P \leq 0.05$) within each variety. Concentrations were expressed as mg g^{-1} of dry weight.

Abbreviation: CTR, control berries (not UV-B treated); UV-B, berries treated with UV-B light; n.s., not significant.

thickness] from Agilent. Single compounds were tentatively identified by comparing the mass spectra with those available in the data system library (NIST 08, National Institute of Standards and Technology, Gaithersburg, MD, USA; 2008). Quantification was performed using the internal standard method and standard solutions of single compounds. The monoterpenes, geraniol, linalool and α-terpineol derivatives, were grouped following the aggregation proposed in D'Onofrio et al.[44] with the additions reported in Palai et al.[46,47] based on their common biosynthetic derivation by terpene synthases from geranyl diphosphate.

Statistical analysis

Within each variety, differences between the control and the UV-B-irradiated berries were assessed by Student's t-test ($P \leq 0.05$). Results are reported as mean $\pm$ standard error (SE). Besides, the VOCs data from each variety were used to perform a canonical discriminant analysis (CDA) in order to emphasize the differences between the control and UV-B treated groups within each grape variety. To highlight the strength of the linear relationship between variables, a CDA-based Pearson's correlation test was performed, comparing the variables considered with the scores of the canonical function arising from the CDA. JMP software (SAS Institute, Inc., Cary, NC, USA) was used to perform all the statistical elaborations.

RESULTS

Berry technological parameters

The grape berries were collected at harvest, whose date was differently established for each variety according to the protocol of the farm corresponding to the day of the year (DOY 251, MOS; DOY 253, ALE; DOY 257, SAN and VER (Table 1). The berry FW before UV-B exposure reflected the peculiar ampelographic features with the highest (2.88 g) and lowest (2.04 g) values measured in VER and ALE berries, respectively (Table 1). After treatment, the berry FW was reduced by 2.82% regardless of treatment, with no significant differences between control and UV-B berries. The TSS content ranging from 23.06 °Brix of SAN UV-B to 26.80 °Brix of ALE CTR (control) (Table 1) and it was not statistically different between treatments, within each variety. The highest TA value (7.95 g L^{-1} tartrate) was measured in ALE CTR, and it was significantly different with respect to ALE UV-B.

Berry primary metabolites

The ^{1}H-NMR spectroscopy was chosen as analytical technique because of the great advantage in short sample preparation and

the fast quantification. NMR is known to be characterized by less sensitivity compared to other analytical methods, such as mass spectroscopy. This is the rationale for the choice of target annotation and quantification on primary metabolites. The spectra of representative grape samples are mainly characterized by the presence of sugars (range δ 3.00–5.50 ppm), mainly glucose and fructose (Supporting Information Fig. S1). Approximately 20 compounds were annotated including organic acids, particularly acetic, malic, syringic, and tartaric acids, and amino acids, in particular alanine, arginine, isoleucine, glutamine, proline, valine, and threonine (Table 2). Using NMR, we were also able to quantify a limited number of highly abundant secondary metabolites, namely catechin and epicatechin. No other compounds were visible within the limit of detection, this led us to choose quantification of phenolic compounds including catechin and epicatechin using HPLC-DAD. The UV-B treatment significantly increased the concentration of acetic acid only in MOS (+56%). Syringic acid was detected only in the red varieties SAN and ALE, while amino acids, arginine and proline were not detected in MOS CTR berries. In addition, arginine was undetectable also in VER, regardless of the treatment. Finally, the content of the amino acid isoleucine decreased following UV-B treatment only in the red varieties SAN (−17%) and ALE (−26%).

Berry phenolic concentration

The total anthocyanin concentration of the red grape varieties SAN and ALE was significantly reduced by 11% and 12%, respectively, after UV-B exposure (Fig. 1(A)). Besides, the tri-substituted and the methoxylated forms were significantly reduced in ALE, particularly petunidin-glucoside (-glucoside, -GS), malvidin-GS, and malvidin-coumaroyl-GS, who showed a 23%, 10%, and 13% decrease compared to the relative control, respectively (Table 3). However, the di-substituted forms, like cyanidin-GS and peonidin-GS, were significantly reduced in SAN berries by 18% and 12%, respectively.

The total flavonol concentration was significantly lower in UV-B-treated berries of VER (13.70 µg g^{-1}) with respect to the CTR ones (16.75 µg g^{-1}) (Fig. 1(B)). Quercetin compounds bounded with different glycosides were significantly reduced in ALE, MOS and VER, as well as isorhamnetin-glucuronide (-glucuronide, -GN) (Table 3). Similarly, kaempferol-galactoside (-galactoside, -GA), kaempferol-GN and kaempferol-GS were reduced in UV-B-exposed VER, MOS and ALE berries, respectively, whereas myricetin was affected by the treatment only in VER. A different pattern was observed in SAN, where the berry total flavonol
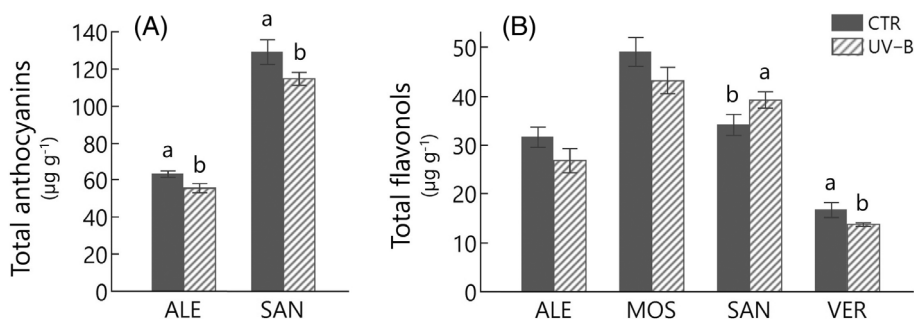

Figure 1. Total anthocyanin (A) and total flavonol (B) concentration (µg g^{-1}) measured in berries of Aleatico (ALE), Moscato bianco (MOS), Sangiovese (SAN) and Vermentino (VER) grapevines (*Vitis vinifera* L.) subjected to postharvest ultraviolet-B (UV-B) treatment. Bars indicate standard deviation of three replicates per treatment. Different letters indicate significant differences respect to control (CTR) after Student's t-test ($P \leq 0.05$) within each variety. Legend: CTR, control berries (not UV-B-treated); UV-B, berries treated with UV-B light.

Table 3. Berry phenolic concentration (μg g^{-1}) measured in berries of Aleatico (ALE), Moscato bianco (MOS), Sangiovese (SAN) and Vermentino (VER) grapevines (*Vitis vinifera* L.) subjected to postharvest ultraviolet-B (UV-B) treatment

Metabolic class	Compound	ALE CTR	ALE UV-B	ALE P	MOS CTR	MOS UV-B	MOS P	SAN CTR	SAN UV-B	SAN P	VER CTR	VER UV-B	VER P
Anthocyanins	Delphinidin_GS	4.17 ± 0.67	2.85 ± 0.31	n.s.	—	—	—	26.73 ± 0.60	24.61 ± 0.72	n.s.	—	—	—
	Cyanidin_GS	5.30 ± 0.12	4.94 ± 0.15	n.s.	—	—	—	16.41 ± 0.57	13.42 ± 0.25	**0.009**	—	—	—
	Petunidin_GS	5.09 ± 0.28	3.92 ± 0.26	**0.04**	—	—	—	27.97 ± 1.37	24.88 ± 0.73	n.s.	—	—	—
	Peonidin_GS	7.43 ± 0.17	7.03 ± 0.29	n.s.	—	—	—	14.16 ± 0.31	12.52 ± 0.21	**0.01**	—	—	—
	Malvidin_GS	19.09 ± 0.51	17.12 ± 0.41	**0.04**	—	—	—	29.95 ± 1.19	27.11 ± 0.50	n.s.	—	—	—
	Peonidin_Ac_GS	0.30 ± 0.00	0.30 ± 0.02	n.s.	—	—	—	0.07 ± 0.02	0.28 ± 0.01	**0.001**	—	—	—
	Malvidin_Ac_GS	0.35 ± 0.03	0.29 ± 0.01	n.s.	—	—	—	0.34 ± 0.02	0.22 ± 0.01	**0.01**	—	—	—
	Peonidin_Caf_GS	1.25 ± 0.08	1.12 ± 0.05	**0.01**	—	—	—	0.24 ± 0.02	0.49 ± 0.01	**<0.001**	—	—	—
	Malvidin_Caf_GS	0.48 ± 0.00	0.52 ± 0.01	**0.04**	—	—	—	0.58 ± 0.06	0.01 ± 0.00	**<0.001**	—	—	—
	Cyanidin_Cum_GS	0.79 ± 0.05	0.98 ± 0.04	n.s.	—	—	—	0.05 ± 0.01	0.04 ± 0.01	n.s.	—	—	—
	Petunidin_Cum_GS	0.65 ± 0.02	0.62 ± 0.03	n.s.	—	—	—	0.11 ± 0.00	0.09 ± 0.00	**0.007**	—	—	—
	Peonidin_Cum_GS	4.51 ± 0.19	3.88 ± 0.17	n.s.	—	—	—	0.32 ± 0.02	0.25 ± 0.01	**0.03**	—	—	—
	Malvidin_Cum_GS	10.79 ± 0.24	9.41 ± 0.36	**0.03**	—	—	—	0.38 ± 0.02	0.33 ± 0.01	n.s.	—	—	—
	3′ 4′	19.58 ± 0.37	18.24 ± 0.66	n.s.	—	—	—	31.24 ± 0.88	27.01 ± 0.46	**0.01**	—	—	—
	3′ 4′ 5′	40.62 ± 0.68	34.73 ± 0.94	**0.007**	—	—	—	86.05 ± 2.95	77.27 ± 1.58	n.s.	—	—	—
	3′ 4′ 5′/3′ 4′	2.08 ± 0.04	1.91 ± 0.06	n.s.	—	—	—	2.76 ± 0.09	2.86 ± 0.06	n.s.	—	—	—
	OH- sub forms	10.26 ± 0.69	8.77 ± 0.40	n.s.	—	—	—	43.18 ± 1.15	38.08 ± 0.61	**0.02**	—	—	—
	OCH3-sub forms	49.94 ± 1.38	44.21 ± 0.99	**0.03**	—	—	—	74.11 ± 2.85	66.20 ± 1.43	n.s.	—	—	—
	Σ acylated	19.12 ± 0.59	17.12 ± 0.64	n.s.	—	—	—	2.09 ± 0.09	1.72 ± 0.04	**0.02**	—	—	—
	Σ not-acylated	41.08 ± 0.59	35.86 ± 0.83	**0.007**	—	—	—	115.20 ± 3.40	102.55 ± 1.80	**0.03**	—	—	—
Flavonols	Myricetin_GN	1.11 ± 0.02	1.08 ± 0.04	n.s.	0.62 ± 0.01	0.53 ± 0.05	n.s.	1.85 ± 0.60	7.29 ± 0.33	**<0.001**	0.22 ± 0.03	0.24 ± 0.03	n.s.
	Myricetin_GS	2.91 ± 0.07	2.80 ± 0.09	n.s.	2.47 ± 0.16	1.89 ± 0.18	n.s.	3.87 ± 0.51	2.36 ± 0.07	**0.001**	2.55 ± 0.08	1.92 ± 0.02	**0.002**
	Quercetin_GN	2.79 ± 0.12	2.20 ± 0.18	n.s.	1.78 ± 0.13	1.68 ± 0.12	n.s.	2.28 ± 1.92	10.83 ± 0.47	**<0.001**	4.44 ± 0.35	3.28 ± 0.11	**0.03**
	Quercetin_GA	4.14 ± 0.04	3.17 ± 0.16	**0.005**	4.03 ± 0.36	5.09 ± 0.16	n.s.	9.13 ± 1.88	2.86 ± 0.36	**0.001**	0.63 ± 0.08	0.61 ± 0.19	n.s.
	Quercetin_GS	2.49 ± 0.11	2.41 ± 0.09	n.s.	7.24 ± 0.32	5.8 ± 0.30	**0.03**	4.00 ± 0.36	4.19 ± 0.30	n.s.	0.61 ± 0.14	0.35 ± 0.05	n.s.
	Quercetin_RT	4.76 ± 0.28	4.59 ± 0.18	n.s.	20.55 ± 0.85	17.49 ± 0.70	n.s.	2.57 ± 0.39	2.33 ± 0.07	n.s.	3.05 ± 0.104	2.20 ± 0.17	**0.01**
	Kaempferol_GA	8.08 ± 0.42	5.95 ± 0.99	n.s.	1.83 ± 0.06	1.66 ± 0.08	n.s.	1.73 ± 0.07	1.94 ± 0.10	n.s.	1.02 ± 0.08	0.40 ± 0.16	**0.03**
	Kaempferol_GN	0.73 ± 0.04	1.05 ± 0.21	n.s.	1.29 ± 0.01	1.01 ± 0.05	**0.006**	1.79 ± 0.70	4.29 ± 0.36	**0.002**	2.44 ± 0.05	3.15 ± 0.07	**0.001**
	Kaempferol_GS	0.86 ± 0.03	0.09 ± 0.03	**<0.001**	3.72 ± 0.05	3.33 ± 0.14	n.s.	4.14 ± 1.40	0.25 ± 0.02	**<0.001**	0.08 ± 0.01	0.07 ± 0.01	n.s.
	Isorhamnetin_GA	0.68 ± 0.08	0.09 ± 0.04	n.s.	1.10 ± 0.04	1.30 ± 0.07	n.s.	0.19 ± 0.07	0.27 ± 0.01	n.s.	0.13 ± 0.04	0.36 ± 0.02	**0.001**
	Isorhamnetin_GN	0.13 ± 0.02	0.59 ± 0.09	**0.009**	1.10 ± 0.03	0.79 ± 0.05	**0.006**	0.24 ± 0.10	0.20 ± 0.04	n.s.	0.46 ± 0.04	0.09 ± 0.01	**0.001**
	Isorhamnetin_GS	0.17 ± 0.07	0.47 ± 0.21	**0.002**	1.46 ± 0.08	1.04 ± 0.06	**0.01**	0.36 ± 0.47	0.36 ± 0.02	n.s.	0.11 ± 0.04	0.09 ± 0.03	n.s.
Other phenols	Catechin	0.14 ± 0.09	0.15 ± 0.04	n.s.	0.13 ± 0.10	0.16 ± 0.11	n.s.	0.29 ± 0.09	0.38 ± 0.10	n.s.	0.10 ± 0.03	0.07 ± 0.03	n.s.
	Epicatechin	0.39 ± 0.26	0.37 ± 0.05	n.s.	0.28 ± 0.13	0.32 ± 0.14	n.s.	0.18 ± 0.02	0.21 ± 0.05	n.s.	0.23 ± 0.02	0.18 ± 0.03	n.s.
	Epicatechin/ Catechin	3.05 ± 0.88	2.56 ± 0.47	n.s.	3.76 ± 3.31	2.78 ± 1.75	n.s.	0.65 ± 0.19	0.61 ± 0.35	n.s.	2.40 ± 0.58	2.60 ± 0.64	n.s.
	Resveratrol	0.08 ± 0.02	0.10 ± 0.01	n.s.	0.11 ± 0.01	0.11 ± 0.01	n.s.	0.18 ± 0.01	0.17 ± 0.01	**0.04**	0.08 ± 0.01	0.05 ± 0.01	n.s.

Note: Values are means of three replicates per treatment ± standard deviation. Statistical significance was determined by Student's *t*-test ($P \leq 0.05$) within each variety.

Abbreviation: GS, glucoside; GN, glucuronide; GA, galactoside; RT, rutinoside; Ace, acetyl; Caf, caffeoyl; Cou, coumaroyl; CTR, control berries (not UV-B treated); UV-B, berries treated with UV-B light; n.s, not significant.
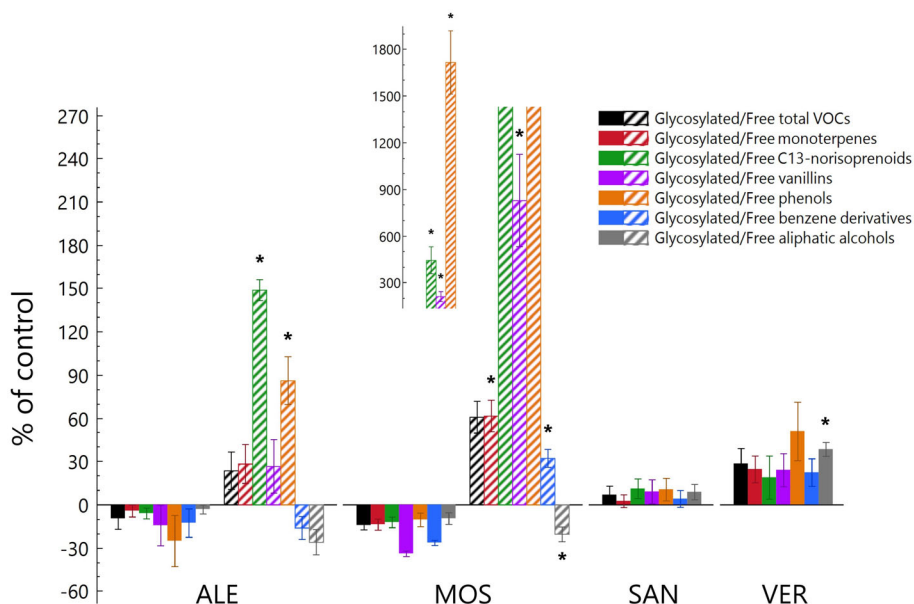
Figure 2. Changes in glycosylated and free VOCs concentration measured in berries of Aleatico (ALE), Moscato bianco (MOS), Sangiovese (SAN) and Vermentino (VER) grapevines (*Vitis vinifera* L.) subjected to postharvest ultraviolet-B (UV-B) treatment. Values are expressed as percentage of those measured in control (CTR). Bars indicate standard deviation of three replicates per treatment. Asterisks indicate significant differences respect to CTR after Student's *t*-test ($P \leq 0.05$) within each variety.
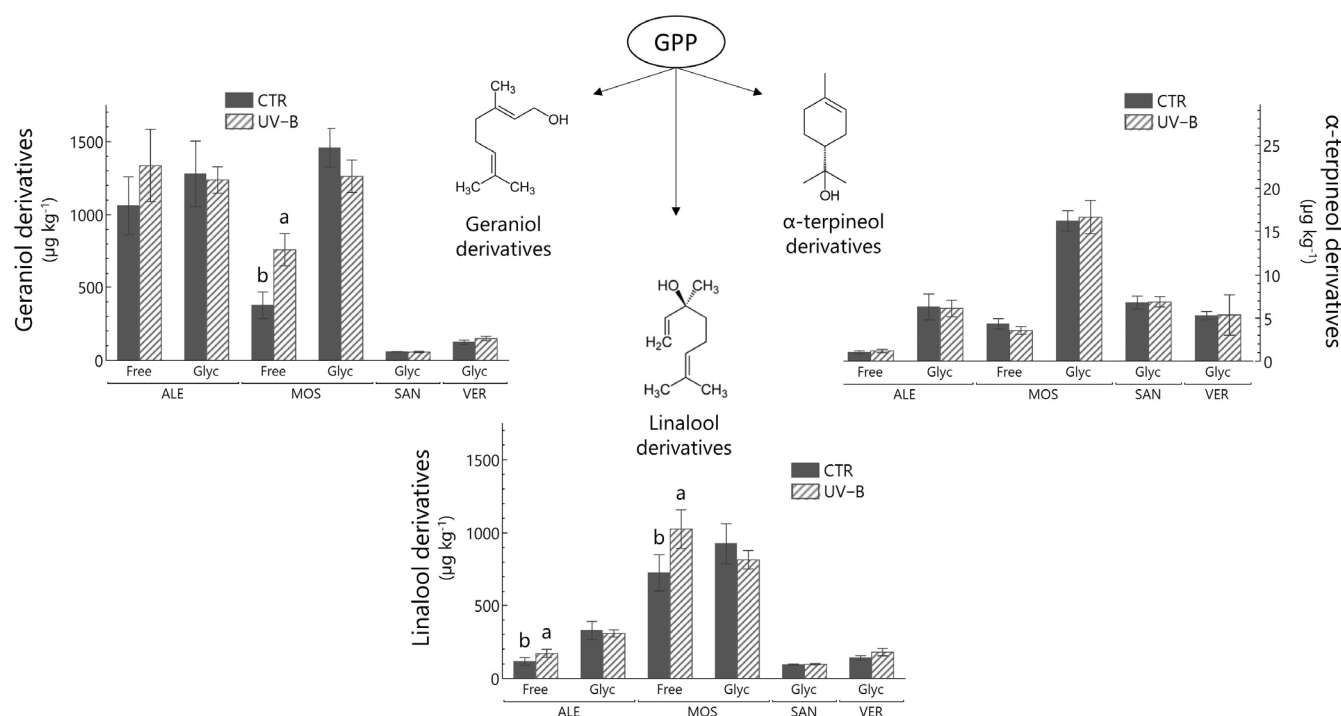
Figure 3. Total free and glycosylated geraniol, linalool and α-terpineol derivatives measured in berries of Aleatico (ALE), Moscato bianco (MOS), Sangiovese (SAN) and Vermentino (VER) grapevines (*Vitis vinifera* L.) subjected to postharvest ultraviolet-B (UV-B) treatment. Bars indicate standard deviation of three replicates per treatment. Different letters indicate significant differences respect to control (CTR) after Student's *t*-test ($P \leq 0.05$) within each variety/ volatile organic compounds (VOCs) form.

concentration increased by 16% after UV-B exposure (Fig. 1(B)). In particular, myricetin-GN, quercetin-GN and kaempferol-GN were significantly enhanced after the treatment (Table 3).

The UV-B effect was less evident on the flavan-3-ols and stilbenes detected (Table 3). No significant differences were reported for catechin and epicatechin, while resveratrol was reduced in

SAN UV-B-exposed berries (0.175 μg g^{-1}) with respect to the CTR ones (0.182 μg g^{-1}).

Berry VOCs

The UV-B largely affect the berry aroma profile, especially the free fraction, which displayed an overall increase due to the treatment
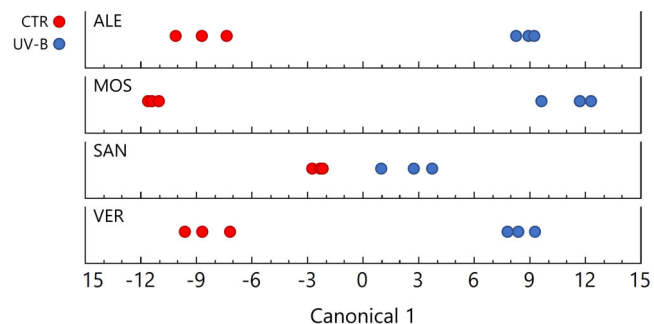
7

Figure 4. Two-dimensional scatterplot of canonical discriminant analysis (CDA) between control and postharvest ultraviolet-B (UV-B)-treated berries of Aleatico (ALE), Moscato bianco (MOS), Sangiovese (SAN) and Vermentino (VER) grapevines (*Vitis vinifera* L.). The CDA was performed using the whole free/glycosylated volatile organic compounds (VOCs) dataset within each grape variety.

Table 4. Pearson's correlation coefficient (*r*) between each free and glycosylated volatile organic compounds (VOCs) detected and the canonical scores for each canonical discriminant analysis (CDA) reported in Fig. 2

Compound	*r*
MOS	
3-methyl-2-buten-1-ol	0.98
6-methoxy-3-methylbenzofuran	0.97
1-octen-3-ol	0.95
1-octadecanol	0.95
ethylhexanol	0.95
2,3-pinanediol	0.94
phenol-2,6-dimethoxy	0.94
β-phenoxyethyl alcohol	0.94
geranic acid	0.94
homovanillic acid	0.94
acetovanillone	0.93
3′,5′-dimethoxyacetophenone	0.93
octanol	0.92
homovanillyl alcohol	0.92
acetosyringone	0.92
2-phenylethanol	0.91
methyl vanillate	0.89
eugenol	0.88
citronellol	0.88
phenol-3,4,5-trimethoxy-	0.88
4-vinylguaiacol	0.88
GS_lilac alcohol C	0.86
GS_1-hexanol	0.85
cis-8-OH-linalool	0.85
lilac alcohol B	0.83
2,6-dimethyl-1,7-octadiene-3,6-diol 2	0.83
geraniol	0.83
nerol	0.82
7-OH-geraniol	0.82
2,6-dimethyl-6-OH-2,7-octadienoic acid	0.81
benzaldehyde	−0.81
GS_zingerone	−0.81
GS_acetosyringone	−0.82
GS_3-oxo-α-damascone	−0.84
GS_3,4,5-trimethoxybenzyl methyl ether	−0.84

Table 4. Continued

Compound	*r*
GS_2,3,4-trimethoxybenzyl alcohol	−0.84
cis-3-hexen-1-ol	−0.84
1-hexanol	−0.85
GS_2,4-methoxyphenylethanol	−0.85
GS_β-citronellal	−0.86
GS_2,4,4-trimethyl-3-oxobuthylcycloexan	−0.87
trans-2-hexenol	−0.89
(*E*)-furanoid linalool ox. A	−0.89
linalool	−0.90
m-methylacetophenone	−0.92
p-cymen-7-ol	−0.92
GS_γ-hydroxyisoeugenol	−0.96
methyl benzoate	−0.98
ALE	
3-methyl −2-buten-1-ol	0.96
lilac alcohol A	0.95
ethylhexanol	0.89
1-octen-3-ol	0.89
phenol-2,6-dimethoxy	0.87
3,4-dimethoxybenzyl alcohol	0.87
linalool	0.83
β-phenoxyethyl alcohol	0.83
GS_guaiacol	0.82
3′,5′-dimethoxyacetophenone	0.81
GS_actinidols B	−0.83
trans-2-hexenol	−0.84
GS_benzenepropanol	−0.87
m-methylacetophenone	−0.99
p-cymen-7-ol	−0.99
VER	
GS_1-hexanol	0.96
GS_4-methyl-3-penten-1-ol	0.94
GS_*cis*-3-hexen-1-ol	0.93
GS_*trans*-2-hexenol	0.84
GS_6,7-dihydro-7-hydroxylinalool	0.83
GS_7-OH-geraniol	0.83
GS_actinidols B	0.83
GS_1,7-octanediol-3,7-dimethyl-	0.81
GS_3,4-dihydro-3-oxo-α-ionol (III)	0.81
GS_3,9-dihydroxy megastigma-5-ene	0.80
GS_3,4-dihydro-3-oxo-α-ionol (I)	0.80
GS_*trans*-8-OH-linalool	0.80
SAN	
GS_epimanool	0.92
GS_3-hydroxy-7,8-dihydro-β-ionol	0.91
GS_octanol	0.90
GS_3,4,5-trimethoxybenzyl methyl ether	0.87
GS_*cis*-8-OH-linalool	0.85
GS_3,4-dimethoxybenzyl alcohol	0.85
GS_methyl vanillate	0.85
GS_3,4-dihydro-3-oxo-α-ionol (II)	0.81

Abbreviation: ALE, MOS, SAN and VER indicate the CDA assessed on Aleatico, Moscato bianco, Sangiovese and Vermentino VOCs dataset, respectively. For each CDA are reported only those VOCs which showed strong correlation ($r < −0.8 \wedge r > 0.8$). 'GS' indicates glycosylated compounds.

(Supporting Information Table S1). In ALE, free C_{13}-norisoprenoids and free phenols were significantly enhanced after UV-B irradiation (+149 and + 86% than CTR; Fig. 2, Table S1). In MOS, this effect was even more evident, and significant increments were observed for free monoterpenes (+62%), free C_{13}-norisoprenoids (+444%), free vanillins (+211%), free phenols (+1814%), and free benzene derivatives (+32%) (Fig. 2, Table S1). Most free phenols and vanillins increased after UV-B treatment in MOS berries, particularly 4-vinylguaiacol (+7046%), eugenol (+161%), homovanillyl alcohol (+128%) and homovanillic acid (+629%). Among free monoterpenes, linalool was significantly higher in UV-B ALE berries (+36%) whereas the contrary was observed in MOS (−54%) (Table S1). Significant increments were also reported for many other free monoterpenes in MOS berries subjected to UV-B, such as citronell, geraniol, 2,6-dimethyl-1,7-octadiene-3,6-diol 2,2,3-pinanediol, cis-8-OH-linalool and geranic acid. The derived compounds from the three key monoterpenes of the methylerythritol 4-phosphate (MEP) pathway were affected by treatment but just in the free form (Fig. 3, Table S1). In particular, the free geraniol and linalool derivatives concentration was significantly higher in MOS UV-B berries with respect to the CTR ones. Similarly, free linalool derivatives had a significantly higher concentration in UV-B (172 µg kg^{-1}) than CTR (117 µg kg^{-1}) ALE berries (Fig. 3, Table S1).

The effect of the treatment on total glycosylated VOCs concentration was not significant but showed a different pattern between varieties: UV-B exposure reduced bounded VOCs in ALE and MOS berries while it slightly promoted their accumulation in SAN and VER (Fig. 2, Table S2). Thus, some glycosylated compounds were significantly lower in UV-B treated MOS and ALE berries, such as γ-hydroxyisoeugenol (−32%), acetosyringone (−29%), 3-oxo-α-damascone (−58%) and β-citronellal (−30%). However, many glycosylates were significantly enhanced by UV-B in SAN and VER berries, such as 1-hexanol, nerol, 6,7-dihydro-7-hydroxylinalool, 7-OH-geraniol and cis-8-OH-linalool (Table S2).

Evaluating the berry total VOCs concentration (glycosylated + free), UV-B differently affected the ratio of glycosylated to free VOCs of ALE to MOS, increasing the ratio between the two fractions, increasing the proportion of free VOCs in most of all the classes of compounds detected (Fig. S2). In particular, after treatment, the free VOCs passing from 34% to 41% (ALE) and from 24% to 38% (MOS) of the total VOCs concentration (Fig. S2).

The CDAs (Fig. 4), corresponding to the different varieties, reported a significant modulation of VOCs due to the UV-B exposure. Particularly, between the varieties analyzed, MOS and SAN showed the greatest and the lowest segregation of VOCs, respectively (Fig. 4). To determine which VOCs are mainly responsible for the segregation between control and UV-B treated groups, for each variety the CDA based Pearson's correlation between each individual VOC and the canonical scores was calculated (Table 4). A total of 30 discriminant VOCs with a positive Pearson's coefficient was identified in MOS and, interestingly, only two were glycosylated compounds. Though monoterpenes were the most abundant discriminant VOCs the highest positive r values were associated to 3-methyl-2-buten-1-ol and 6-methoxy-3-methylbenzofuran (r values of 0.98 and 0.97, respectively), while the highest negative r values were associated to methyl benzoate and glycosylated (GS)_γ-hydroxyisoeugenol (r values of −0.98, and − 0.96, respectively).

In ALE, the most discriminating compounds were 3-methyl-2-buten-1-ol, (r value of 0.96), lilac alcohol A (r value of 0.95),

phenol-2,6-dimethoxy (r value of 0.87), and 3,4-dimethoxybenzyl alcohol (r value of 0.87). The negative strongest correlations were found for p-cymen-7-ol and the two benzene derivatives m-methylacetophenone and GS_benzenepropanol.

For both VER and SAN varieties the Pearson's analysis highlighted only positive correlations between the canonical scores and the variables measured and the segregation was mainly due to glycosylated VOCs (Table 4). Specifically, the aliphatic alcohols showed the strongest correlation for VER, whereas three C_{13}-norisoprenoids (GS_epimanool; GS_3-hydroxy-7,8-dihydro-β-ionol; GS_3,4-dihydro-3-oxo-α-ionol (II); r values of 0.92, 0.91, and 0.81, respectively) and two vanillins (GS_3,4,5-trimethoxybenzyl methyl ether and GS_methyl vanillate; r values of 0.87 and 0.85, respectively) showed the strongest correlation for SAN.

DISCUSSION

Postharvest is critical for non-climacteric fruits such as grapes, because of the progression of catabolic reactions that modify the concentration of compounds considered important for quality.

The application of UV-B treatments, thanks to the influence played by this radiation on many metabolic pathways,[48-52] could boost the synthesis of important metabolites, and re-equilibrate their concentration. The effects of UV radiation on grape berries have been studied during preharvest, leading to different outcomes also depending on the stage at which the treatment was applied.[20,53,54] Gregan et al.[20] found a general decrease of amino acids of Sauvignon blanc grapes following deprivation of the UV-B or all UV wavelengths by applying shielding plastics screens at 5 weeks pre-veraison up to harvest, indicating the importance of this radiation for the synthesis of amino acids. In our research, the results on primary metabolites highlighted the absence of UV-B influence on the concentration of total free amino acids as well as most of the other primary metabolites identified. These findings are in accordance with those reported by Martínez-Lüscher et al.[55] on Tempranillo grapes irradiated in planta with two UV-B doses (5.98 kJ m^{-2} d^{-1} or 9.66 kJ m^{-2} d^{-1}). In addition, they found changes in some individual amino acids, also depending on the stage of UV-B application, as, for example, for proline. In our samples, proline was unchanged in all varieties, except for MOS, where it was found only in UV-B treated grapes, indicating a genotype-dependent response. According to Zoecklein et al.[56] arginine and glutamine are the preferred sources for de novo synthesis of amino acids by yeast during wine fermentation, so changes in their levels, as the appearance of arginine in UV-B treated MOS, could be important for yeast metabolism and the proper progression of the alcoholic fermentation.

Between secondary metabolites, it has been shown that light conditions play a crucial role in the metabolism of phenolic compounds.[57] In accordance with the findings of Martínez-Lüscher et al.[55] the concentration of total anthocyanins was negatively affected by UV-B irradiation. Anthocyanins are reported to be less reactive to UV radiation than to photosynthetically active radiation (PAR),[58] and less reactive to UV-B than UV-A radiation,[59] so the detected decrease we observed could derive from anthocyanin degradation due to UV-B derived oxidative stress not flanked by up-regulation of anthocyanin biosynthesis. Only peonidin-ac-GS, peonidin-caf-GS, in SGV, malvidin-caf-GS and cyanidin-cum-GS in ALE were positively influenced by the treatment, showing an increase probably due to internal rearrangements of the

metabolic pathways responsible for the addition of the substituents. However, in accordance with Martínez-Lüscher *et al.*[55] UV-B application did not differentially affect specific anthocyanin groups (di- or tri-substituted, methylated or esterified with hydroxycinnamic acids). The UV-B influence on berries anthocyanins is extremely dependent on genetic and environmental factors, in addition to the UV-B doses applied, so that increases,[24,60] decreases,[12] or invariance[10] have been reported.

The concentration of flavonols was unaffected by UV-B in ALE and MOS and undergoing an increase or decrease in SAN and VER variety, respectively. In accordance with our findings on SAN variety, an increase of total flavonols was reported by Martínez-Lüscher *et al.*[55] and Del-Castillo-Alonso *et al.*[54] on Tempranillo grapes exposed to UV radiation in preharvest. Interestingly, the flavonol increase in SAN grapes was due to the marked increment of the GN forms of myricetin, quercetin and kaempferol, while the GS and GA forms were negatively influenced or unaffected by the UV-B treatment. Conversely, the decrease of total flavonols detected in VER was more generalized and less linked to a specific sugar moiety. Differently from our results, Liu *et al.*[22] reported an accumulation of glycosylated flavonols (mainly quercetin and kaempferol) in Sauvignon blanc berries under UV-B transmitting screens. Differences are likely due to genotype-related susceptibility to UV-B as well as to the UV-B dose and mode of application (preharvest *versus* postharvest, screening of solar radiation *versus* lamp irradiation).

Sunlight exposure and composition are also important factors influencing grape aroma profile.[15,17,61] In this study we examined both free and glycosylated VOCs, in four varieties with different aroma traits: ALE and MOS are 'aromatic' varieties characterized by free VOCs concentration higher than the perception threshold, while SAN and VER are so-called 'neutral' varieties with a free VOCs concentration lower than the perception threshold.[62] The effect of postharvest UV-B exposure on berry VOCs was consistent with these peculiarities. The free and glycosylated fraction was enhanced and reduced, respectively, in ALE and MOS berries, whereas on the contrary, glycosylated VOCs increased in UV-B treated SAN and VER berries. Therefore, the free/glycosylated compounds ratio characterizing the aroma profile of ALE and MOS was modified by the increment of free VOCs which are the key fraction in these aromatic varieties. This finding is also particularly relevant for table grape varieties where the free VOCs fraction is perceived by the customers as one of the most important quality features.

Free phenols were the most enhanced compounds in MOS UV-B berries, particularly β-phenoxyethyl alcohol and 4-vinylguaiacol. This result could be coherently explained considering free phenols share the biosynthetic origin with other berry phenols such as flavanols, flavonols and phenolic acids, which in turn increased in berries exposed to UV-B.[54,55,60]

Free C_{13}-norisoprenoids were significantly increased in both ALE and MOS UV-B berries as well. In agreement to our results, β-damascenone concentration was reported depressed in Cabernet Sauvignon berries subjected to on-season UV radiation attenuation.[63] A general effect of sunlight exposure on berry C_{13}-norisoprenoids was widely observed in Riesling and Cabernet Sauvignon juice and wine, where sun-exposed clusters showed an increment of 1,1,6-trimethyl-1,2-dihydronaphthalene and vitispirane.[64,65] Since norisoprenoids are derived from carotenoids which fill a key role under high light exposure by scavenging singlet oxygen or quenching triplet state chlorophyll,[66] we can hypothesize that the higher concentrations of C_{13}-norisoprenoids

induced by UV-B treatments could be related with a photoprotection function.

Berry monoterpenes were proposed as involved in grapevine tolerance to abiotic stress, particularly UV-B radiation under field conditions.[67,68] A previous study found that high UV exposure induced the accumulation of diterpenes in grapevine vegetative tissue and their contribution in membrane stability has been hypothesized.[49] In this experiment we observed a significant increment of monoterpenes in MOS UV-B berries, particularly free linalool derivatives and free geraniol derivatives. Similarly, ALE UV-B berries showed significantly higher values of free linalool and lilac alcohol A. It is worth noting as also some SAN and VER glycosylated monoterpenes were significantly enhanced by UV-B, particularly nerol, 6,7-dihydro-7-hydroxylinalool, 7-OH-geraniol (VER) and *cis*-8-OH-linalool (SAN). Even if not always significant, the widespread boost of monoterpenes in all the varieties examined, support the hypothesis of their active role in protection against UV-B exposure. Similar effects were also reported in field experiments, applying or shading UV-B prior to harvest. On Pinot noir, the berry concentrations of free nerol and geraniol were significantly higher in berries subjected to UV-B boosting using acrylic sheets.[69] Similarly, the concentration of free geraniol and limonene increased following low UV-B irradiance both *in vitro* and field conditions in Malbec.[53] Contrasting results were reported in Shiraz berries, where a small reduction of total monoterpenes concomitantly with a higher concentration of sesquiterpenes was observed in UV-attenuated treatment from berry pea-size to harvest with respect to a naturally exposed control.[70] Some monoterpene enzyme transcripts coding for terpene synthases such as linalool synthase, were induced by solar UV radiation in Tempranillo,[71] remarking the direct biosynthetic effect of UV on terpenes accumulation when applied during the vegetative season.

Overall, these findings reported in the literature clearly show that UV radiation exposure prior to harvest affected berry VOCs by modulating their biosynthesis, consistently with the fact that UV-B can directly affect the VOCs biosynthetic pathways, which are primarily activated during ripening.[62,72] Nonetheless, to the best of our knowledge, these are the first findings indicating that also postharvest UV-B treatment can affect the concentration of berry free and glycosylated VOCs, albeit the fact that grape is a non-climacteric fruit. In particular, the free VOCs increment observed in ALE and MOS berries subjected to postharvest UV-B appears to be caused by the aglycones released from the corresponding glycosylated compound, which was then reduced. However, it is also worth noting that for some VOCs, particularly monoterpenes, we found a higher concentration of their total amount (free + glycosylated compounds) leading us to speculate an *ex novo* biosynthesis induced by UV-B.

In conclusion, the new insights provided in this study highlight as the UV-B radiation can modulate berry primary and secondary metabolites even when applied after harvest. These findings suggest the potential use of postharvest UV-B treatment to improve the aroma profile of grape, without negatively impacting the primary metabolism and the commercial quality of berries. Moreover, a genotype-related modulation of secondary metabolites, particularly VOCs, has been observed, thus making it necessary to deepen the knowledge on the UV-B triggered effect on other varieties. Since the grape cultivars used in this study are specifically addressed for wine making, next step will be to evaluate whether the observed changes, especially in terms of modification of the aroma profile, will be maintained in the resulting wine.

Also, considering the relevance of berry VOCs in table grapes, further findings on this varieties may be interesting to improve the commercial value of the fruits.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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