OENO One
VINE AND WINE OPEN ACCESS JOURNAL
By IVES International Viticulture & Enology Society

macrowine 2021 Virtual June 23 - 30

DOI: https://doi.org/10.20870/oeno-one.2022.56.3.5393

OPEN ACCESS

ORIGINAL RESEARCH ARTICLE

Approaches to the classification of wine aroma ageing potential. Applications to the case of terpenoids in Valpolicella red wines

Giovanni Luzzini[1], Davide Slaghenaufi[1] and Maurizio Ugliano[1*]

[1] University of Verona, Department of Biotechnology, Villa Lebrecht, via della Pieve 70, San Pietro in Cariano, 37029, Italy

This article is published in cooperation with Macrowine 2021, 23-30 June 2021.

*correspondence:
maurizio.ugliano@univr.it

Associate editor:
Doris Rauhut

Received:
31 January 2022

Accepted:
1 July 2022

Published:
4 August 2022

ABSTRACT

Valpolicella is an Italian wine-producing region famous for premium red wines. Valpolicella wines often undergo a minimum ageing period between 1 and 4 years, according to the wine type. Therefore, identifying relevant types of aroma precursors and the related chemical mechanisms behind aroma development during ageing is of great importance for Valpolicella wines. This study assessed free and glycosidically-bound terpene compounds of Valpolicella wines as potential precursors of different ageing-related terpenes using a model ageing experimental approach. Results showed that terpene profiles changed substantially with ageing. The occurrence of cyclic terpenes (*p*-menthane-1,8-diol, 1,8-cineole and 1,4-cineole) was mostly associated with the content of free monoterpenols, linalool in particular, in young wines, whereas glycosylated monoterpenols appeared to play a minor role. Accumulation of cineoles was strongly modulated with pH; in fact, low pH values promoted higher cineole yields.

KEYWORDS: Wine ageing, Valpolicella red wines, cineoles, terpenes, linalool

INTRODUCTION

Valpolicella is a region located in the northeast of Italy, in the province of Verona, where the red wines Valpolicella Superiore, Ripasso, Recioto and the most famous Amarone are produced. The grape varieties used to produce these wines are mainly Corvina and Corvinone and a range of minor varieties (MIPAAF, 2019). Valpolicella wines are characterised by cherry, pepper, spicy, tobacco and balsamic notes, among other aromas. (Accordini, 2013; Bellincontro *et al*., 2016; D'Agata, 2019). Depending on the wine type, a cellar ageing period comprised of one and four years is required by Protected Designation of Origin (PDO) regulation, after which the wine can be released on the market. During ageing, the sensory characteristics of red wine, including its aroma, undergo dramatic changes, with a decrease in fresh fruity attributes and an increase of more complex ripe fruit, dry fruit, tobacco and balsamic aromas (Rapp and Mandery, 1986; McKay *et al*., 2010; Moio, 2016; Ugliano, 2013). Concerning the latter attributes, recent studies on Valpolicella wines highlighted the potential relevance of different terpenes and norisoprenoids (Slaghenaufi and Ugliano, 2018). While some chemical mechanisms underlying sensory evolution are well known (Antalick *et al*., 2014; Díaz-Maroto *et al*., 2005; Tarasov *et al*., 2020; Skouroumounis and Sefton, 2000), such as esters hydrolysis, acid-catalysed formation non-megastigmane norisoprenoids or oxidation-driven accumulation of aldehydes, others are still poorly understood. Among these, in the case of wines from non-aromatic varieties such as Valpolicella reds, the relative contribution of different forms of various terpenoids and norisoprenoids (e.g., free or glycosylated) requires further investigation (Slaghenaufi *et al*., 2019).

Among the terpenes potentially contributing to red wine aroma, various floral monoterpenes, as well as the balsamic cyclic terpenes 1,4-cineole and 1,8-cineole, have been reported (Atalick *et al*., 2015). In the case of Valpolicella wines, some of these were shown to vary significantly according to grape geographical origin, variety and fermentation practices (Slaghenaufi *et al*., 2019; Luzzini, 2021a; Luzzini *et al*., 2021b; Luzzini *et al*., 2021c). In wines from non-aromatic varieties, monoterpene alcohols are largely formed during fermentation from the enzymatic hydrolysis of glycosidic precursors (Ugliano *et al*., 2006; Slaghenaufi *et al*., 2019). Conversely, cyclic terpenes, such as cineoles, appear to be mostly associated with ageing (Fariña *et al*., 2005; Slaghenaufi and Ugliano, 2018), and studies in model systems have shown the existence of complex chemical relationships between these compounds and monoterpene alcohols at wine pH (Slaghenaufi *et al*., 2019).

At the same time, acid-catalysed hydrolysis of glycosidic precursors can release various terpene compounds some of which can undergo further degradation reactions at wine pH (Skouroumounis and Sefton, 2000). Overall, the terpene content of aged red wine is given by the balance between acid-driven formation and degradation reactions. The formation of a carbocation intermediate plays a central role in these reactions, giving rise to a wide range of reaction products with different aromatic characteristics (Wedler *et al*., 2015). Some authors have recently linked the occurrence of balsamic notes in aged wines to the formation of cineoles from terpene precursors (Slaghenaufi and Ugliano, 2018; Poitou *et al*., 2017; Antalick *et al*., 2015; Fariña *et al*., 2015).

This work aimed to investigate the evolution of the Valpolicella wines terpenes pool during ageing, focusing on the compositional factors of young wines determining the chemical patterns of aged wines.

MATERIALS AND METHODS

1. Wine samples

Wines of the two main grape varieties of the Valpolicella appellation, Corvina and Corvinone, were used in the study. The wines were obtained from grapes harvested in mid-September during the 2017, 2018 and 2019 vintages in five different vineyard parcels belonging to the same winery and located in the Valpolicella region. The same five parcels were samples during the three vintages. A total of 30 wines, 15 Corvina (W1-W15) and 15 Corvinone (W16-W30), were obtained for this study. All vineyards had the same clone-rootstock combination. Grapes were harvested and vinified from 13 to 24 September in 2017, 17 September to 1 October in 2018 and 25 September to 14 October 2019. Harvest took place following the schedule established by the winery owning the vineyards so that grapes were processed in agreement with standard winery evaluation of maturity. After manual destemming, berries were randomised to obtain batches of 7 kg each. From each batch, 3.5 kg were taken, hand crushed with 100 mg/kg of potassium metabisulphite and put into a 5 L glass vessel. Fermentations of fresh grapes musts were carried out in duplicate with *Saccharomyces cerevisiae* AWRI 796 (AB Mauri, Camellia, Australia). Active dry yeast of each commercial starter was rehydrated in water at 37 °C for 15 min, and then 7.5 mL of each culture (100 g/L) was used to inoculate individual grape batches. Fermentations were carried out at 22 ± 1 °C, with the cap being broken twice a day by gently pressing it down skins with a steel plunger and density and temperature monitored daily. Upon completion of alcoholic fermentation (glucose-fructose < 2 g/L), wines were pressed, cold settled and then clarified by centrifugation at 4500 rpm for 15 minutes at 5 °C (Avanti J-25, Beckman Coulter, California, USA). Potassium metabisulphite was added to a final free SO_2 concentration of 25 mg/L, after which the wines were bottled in 330 mL glass bottles with crown caps.

2. Standard oenological analyses

Glucose-fructose, polyphenols, acetic acid, and total acidity (expressed in grams of tartaric acid) were analysed using a Biosystems Y15 multiparametric analyser (Sinatech, Fermo, Italy). For each parameter, a specific kit (Sinatech, Fermo, Italy) was used. Ethanol was analysed with an Alcolyzer DMA

4500 (Anton Paar, Graz, Austria). The pH was evaluated with a Crison Basic 20+ pHmeter (Barcelona, Spain).

3. Model ageing protocol

An accelerated ageing protocol was employed based on the procedure described by Slaghenaufi *et al*. (2019). 115 mL of wine was placed in glass vials and crimped, leaving mg/L of oxygen. Vials were sealed with epoxy resin to prevent any oxygen ingress, and sample vials were placed at 16 °C (labelled 'young'-T16) and 40 °C (labelled 'aged'-T40) for 30 days.

4. Analysis of volatile compounds

For quantification of terpenes, SPME extraction followed by GC-MS analysis was used, following the procedure described by Slaghenaufi and Ugliano (2018). 5 µL of internal standard 2-octanol (4.2 mg/L in ethanol) are added to 5 mL of wine diluted with 5 mL of deionised water in a 20 mL glass vial. 3 g of NaCl are added prior to GC-MS analysis. Samples were equilibrated for 1 min at 40 °C. Subsequently, SPME extraction was performed using a 50/30 µm divinylbenzene–carboxen–polydimethylsiloxane (DVB/CAR/PDMS) fibre (Supelco, Bellafonte, PA, USA) exposed to sample headspace for 60 minutes. GC-MS analysis was carried out on an HP 7890A (Agilent Technologies) gas chromatograph coupled to a 5977B quadrupole mass spectrometer, equipped with a Gerstel MPS3 autosampler (Mülheim/Ruhr, Germany). Separation was performed using a DB-WAX UI capillary column (30 m × 0.25, 0.25 µm film thickness, Agilent Technologies) and helium as carrier gas at 1.2 mL/min of constant flow rate. GC oven was programmed as follows: started at 40 °C for 3 min, raised to 230 °C at 4 °C/min and maintained for 20 min. Mass spectrometer operated in electron ionisation (EI) at 70 eV with ion source temperature at 250 °C and quadrupole temperature at 150 °C. Mass spectra were acquired in SIM mode. A calibration curve was prepared for each analyte using seven concentration points and three replicate solutions per point in wine. 5 µL of internal standard 2-octanol (4.2 mg/L in ethanol) were added to each calibration point, which was then submitted to SPME extraction and GC-MS analysis as described for the samples. Calibration curves were obtained using Chemstation software (Agilent Technologies, Inc.) by linear regression, plotting the response ratio (analyte peak area divided by internal standard peak area) against concentration ratio (added analyte concentration divided by internal standard concentration).

For quantification of *p*-menthane-1,8-diol, solid-phase extraction (SPE) followed by GC-MS analysis was used, following the procedure described by Slaghenaufi *et al.* (2019). 100 µL of internal standard 2-octanol (4.2 mg/L in ethanol) are added to samples prepared with 50 mL of wine and diluted with 50 mL of deionised water. Samples were loaded on a BOND ELUT-ENV, SPE cartridge (Agilent Technologies, USA) previously activated with 20 mL of dichloromethane, 20 mL of methanol and equilibrated with 20 mL of water. After sample loading, the cartridges were washed with 15 mL of water. *P*-Menthane-1,8-diol was eluted with 10 mL of dichloromethane, which was then concentrated under a gentle nitrogen stream to 200 µL prior to GC injection. After elution of *p*-menthane-1,8-diol, glycosidic precursors were eluted with 20 mL of methanol for enzymatic hydrolysis, which was performed as described by Slaghenaufi *et al*. (2019). The extracts were transferred in a glass flask and evaporated under vacuum using a Büchi R-215 Rotavapor System (Büchi, Switzerland), redissolved in 5 mL of citrate buffer (pH 5) and added with100 mg of polyvinylpolypyrrolidone (PVPP) and 200 µL of enzyme solution AR2000 (70 mg/mL in citrate buffer). Samples were incubated at 37 °C overnight. Liberated aglycones were extracted as free volatile compounds using the SPE protocol described above. GC–MS analysis was carried out on an HP 7890A (Agilent Technologies) gas chromatograph coupled to a 5977B quadrupole mass spectrometer, equipped with a Gerstel MPS3 autosampler (Mülheim/Ruhr, Germany). Separation was performed using a DB-WAX UI capillary column (30 m × 0.25, 0.25 µm film thickness, Agilent Technologies) and helium as carrier gas at 1.2 mL/min of constant flow rate. GC oven was programmed as follows: started at 40 °C for 3 min, raised to 230 °C at 4 °C/min and maintained for 20 min. Mass spectrometer operated in electron ionisation (EI) at 70 eV with ion source temperature at 250 °C and quadrupole temperature at 150 °C. Mass spectra were acquired in SIM mode.

Calibration curves were prepared using seven concentration points and three replicate solutions per point in model wine (12 % v/v ethanol, 3.5 g/L tartaric acid, pH 3.5) 100 µL of internal standard 2-octanol (4.2 mg/L in ethanol) were added to each calibration solution, which was then submitted to SPE extraction and GC-MS analysis as described for the samples. Calibration curves were obtained using Chemstation software (Agilent Technologies, Inc.) by linear regression, plotting the response ratio (analyte peak area divided by internal standard peak area) against concentration ratio (added analyte concentration divided by internal standard concentration). Retention indices, quantifying and qualifier ions are reported in Supplementary 1.

5. Statistical analysis

One-way ANOVA and Hierarchical Cluster Analysis (HCA) of chemical data have been performed using XLSTAT 2017 (Addinsoft SARL, Paris, France). Heatmap has been performed with MetaboAnalyst 5.0 (http://www.metaboanalyst.ca, accessed on 16 November 2021) created at the University of Alberta, Edmonton, AB, Canada.

RESULTS AND DISCUSSION

The main oenological parameters of wines are shown in Table 1. Relatively wide ranges were observed for all parameters, reflecting the fact that grapes of different varieties, vintages and geographical origins were employed for winemaking. pH values ranged from 2.77 to 3.31, whereas total acidity from 6.5 to 10 g/L and ethanol from 10.2 to 13.9 % v/v. Acid acetic content was generally below 0.4 g/L.

Terpene content of the young (T16) wines are shown in Table 2 and Supplementary 2–4, indicating rather variable terpene

TABLE 1. Enological parameters of young wines.

	Grape variety	Vintage	Total acidity (g/L)		pH		Acetic acid (g/L)		Ethanol (% v/v)	
			mean	sd	mean	sd	mean	sd	mean	sd
W1	Corvina	2017	8	0.1	3.15	0.01	0.18	0.01	12.7	0.3
W2	Corvina	2017	7.9	0.1	3.31	0.01	0.15	0.01	10.9	0.6
W3	Corvina	2017	7.1	0.1	3.19	0.01	0.31	0.01	13.9	0.5
W4	Corvina	2017	8.1	0.1	3.23	0.01	0.39	0.02	12.2	0.1
W5	Corvina	2017	7.8	0.1	3.19	0	0.23	0	12.7	0
W6	Corvina	2018	8.4	0.2	3.22	0.03	0.28	0.04	11.7	0.2
W7	Corvina	2018	7.3	0.4	2.88	0.01	0.39	0.04	10.7	0.3
W8	Corvina	2018	9	0.1	2.96	0.02	0.32	0.02	13.5	0.4
W9	Corvina	2018	8.2	0.1	3.25	0.02	0.26	0.14	12.3	0.1
W10	Corvina	2018	8.8	0.3	2.77	0	0.36	0.1	11.7	0.1
W11	Corvina	2019	7.5	0.2	3.3	0.01	0.25	0.07	10.6	0.2
W12	Corvina	2019	8.2	0.2	2.98	0.01	0.29	0.08	10.6	0
W13	Corvina	2019	8.6	0.3	2.88	0.01	0.17	0.04	12.8	0.2
W14	Corvina	2019	6.5	0.2	3.29	0.01	0.32	0.05	13.9	0.3
W15	Corvina	2019	9.2	0.3	2.89	0	0.32	0.1	12.6	0.2
W16	Corvinone	2017	8.9	0.1	3.21	0.01	0.23	0.01	11.4	0.2
W17	Corvinone	2017	7.4	0.1	3.23	0.02	0.15	0.03	11.2	0.3
W18	Corvinone	2017	7.1	0.1	3.19	0.01	0.19	0	11.4	0
W19	Corvinone	2017	8.1	0.1	3.29	0.02	0.25	0.12	12.8	0.5
W20	Corvinone	2017	8.3	0.1	3.24	0	0.1	0	11.9	0.2
W21	Corvinone	2018	9.7	0.2	3.05	0.02	0.28	0.02	13.7	0.1
W22	Corvinone	2018	6.9	0.3	3.11	0.02	0.24	0.03	10.3	0.2
W23	Corvinone	2018	7.5	0.2	3.23	0.04	0.31	0.01	11.2	0.3
W24	Corvinone	2018	8.3	0.2	3.26	0.04	0.32	0.06	10.3	0.4
W25	Corvinone	2018	8.3	0.1	2.94	0	0.24	0	12.3	0.6
W26	Corvinone	2019	10	0.1	2.97	0.02	< LOQ1		11.7	0.2
W27	Corvinone	2019	8.6	0	3.04	0.01	0.18	0.04	10.2	0.2
W28	Corvinone	2019	7.3	0.1	3.18	0.01	0.13	0.05	10.8	0.4
W29	Corvinone	2019	8.6	0.2	3.12	0.01	0.17	0.04	11.7	0.2
W30	Corvinone	2019	9.3	0.1	2.96	0	0.29	0	12.7	0.3

Values are mean results of two replicates.
[1]LOQ of acetic acid is 0.03 g/L.

profiles across the sample set. Corvina wines were generally richer than Corvinone. In comparison with data reported in the literature (Black *et al*., 2015; Slaghenaufi *et al*., 2022), the contents of the various monoterpene alcohols were relatively high, whereas cyclic and by-cyclic terpenes were generally detected in much lower concentrations.

Glycosylated forms of various monoterpene alcohols were also observed (Table 2 and Supplementary 2–4), the most abundant being linalool, geraniol and nerol. In this case, Corvinone showed higher content compared to Corvina.

Terpene profiles were strongly affected by ageing (Table 2 and Supplementary 2–4), T40 samples), generally showing a decrease in the concentration of monoterpene alcohols such as linalool, β-citronellol and geraniol and an increase in linalool oxides and various cyclic and bi-cyclic terpene and diols. However, as shown in Figure 1a,b, patterns of terpene evolution during ageing were rather complex and appeared to cluster according to different trends. In the case of Corvina, young wines were grouped in two main clusters, one (containing samples W1-5 and W12-15) associated with higher content of linalool, limonene and, in W12-15, also of α-terpineol and γ-terpinene, the other (containing samples W6-11) with higher content of geraniol and β-citronellol. Aged samples (T40) were also separated into two clusters, which only in part corresponded to those of young wines. Indeed, one major cluster included W1, 2, 3, 5, 12, 13, 15 and was characterised by a higher content of linalool oxides, cineoles, terpinen 4-ol and *p*-methane-1,8-diol, while the second cluster (W4 and W6-10) showed moderate levels of cineoles. Moreover, in the case of Corvinone, two clusters were observed for young wines, one (W21-24) associated

TABLE 2. Minimum, maximum, mean content, standard deviation of quantified terpenes and p-values (ANOVA) between T16 and T40 wines.

				T16				T40			
			p-value	min	max	mean	s.d.[1]	min	max	mean	s.d.
				(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)
Corvina	Free terpenes	*p*-Menthane-1,8-diol	**< 0.0001**	0.00	2.15	0.22	0.60	0.00	29.27	12.38	8.98
		cis-Linalool oxide	0.585	0.00	6.91	1.64	2.43	0.00	21.37	4.57	7.43
		trans-Linalool oxide	0.597	0.00	3.26	0.91	1.33	0.00	12.39	2.31	3.95
		Limonene	**0.002**	0.19	2.57	1.12	0.61	0.16	1.01	0.63	0.23
		1,4-Cineole	**< 0.0001**	0.00	0.11	0.02	0.03	0.00	0.25	0.14	0.06
		1,8-Cineole	**< 0.0001**	0.00	0.26	0.08	0.09	0.00	0.69	0.28	0.17
		p-Cymene	**0.014**	0.07	0.39	0.23	0.07	0.07	0.50	0.20	0.09
		Linalool	**< 0.0001**	6.95	57.82	30.25	15.57	2.72	29.66	10.33	7.49
		Terpinen-4-ol	0.290	0.00	1.17	0.51	0.30	0.14	1.04	0.60	0.28
		α-Terpineol	0.506	1.15	27.41	8.77	7.51	2.00	33.25	10.22	8.77
		β-Citronellol	**< 0.0001**	2.47	21.14	9.62	5.86	0.38	8.69	3.48	2.86
		Nerol	**< 0.0001**	0.00	1.47	0.27	0.41	0.00	0.00	0.00	0.00
		Geraniol	**< 0.0001**	1.14	7.64	3.39	1.87	0.00	0.83	0.13	0.23
	Glycosidically bound terpenes	cis-Linalool oxide	0.507	0.00	5.30	1.44	1.37	0.00	5.30	1.18	1.90
		trans-Linalool oxide	**0.054**	0.00	3.61	1.52	1.29	0.00	2.92	0.62	0.90
		Linalool	**< 0.0001**	0.00	13.00	3.10	3.69	0.00	1.43	0.27	0.44
		α-Terpineol	**< 0.0001**	0.34	2.02	1.10	0.54	0.04	0.72	0.22	0.22
		β-Citronellol	**< 0.0001**	0.43	3.71	1.63	0.84	0.01	0.85	0.30	0.25
		Nerol	**< 0.0001**	3.10	17.91	9.05	3.21	0.77	3.10	1.42	0.70
		Geraniol	**< 0.0001**	2.67	37.45	20.48	6.95	1.11	4.12	2.38	0.67
		p-Cymene	**0.001**	0.00	0.12	0.03	0.04	0.00	0.00	0.00	0.00
Corvinone	Free terpenes	*p*-Menthane-1,8-diol	**< 0.0001**	0.00	0.01	0.00	0.00	0.00	11.15	3.62	3.44
		cis-Linalool oxide	**< 0.0001**	0.00	6.92	1.01	1.86	0.00	10.64	4.58	3.21
		trans-Linalool oxide	**0.012**	0.00	1.23	0.27	0.46	0.00	8.42	2.08	2.82
		Limonene	**0.012**	0.17	0.95	0.48	0.21	0.19	0.75	0.36	0.14
		1,4-Cineole	**< 0.0001**	0.00	0.03	0.00	0.01	0.00	0.16	0.04	0.04
		1,8-Cineole	**< 0.0001**	0.00	0.06	0.01	0.02	0.00	0.34	0.13	0.07
		p-Cymene	**0.040**	0.00	0.31	0.13	0.07	0.05	0.18	0.10	0.03
		Linalool	**< 0.0001**	3.71	26.89	14.48	6.91	0.62	18.84	6.36	4.96
		Terpinen-4-ol	**0.016**	0.13	0.85	0.33	0.17	0.21	0.60	0.37	0.10
		α-Terpineol	**0.027**	1.09	17.46	4.44	3.81	2.31	14.02	5.92	3.66
		β-Citronellol	**< 0.0001**	2.45	18.23	8.66	4.85	0.40	8.81	3.58	2.28
		Nerol	**0.002**	0.00	0.96	0.30	0.36	0.00	3.15	0.33	0.87
		Geraniol	**< 0.0001**	0.35	6.25	2.72	1.70	0.00	0.96	0.17	0.29
	Glycosidically bound terpenes	cis-Linalool oxide	0.790	0.00	3.30	0.92	0.92	0.06	1.74	0.52	0.60
		trans-Linalool oxide	0.882	0.00	3.06	0.92	0.95	0.05	1.77	0.57	0.65
		Linalool	**< 0.0001**	0.00	47.77	12.14	13.57	0.00	5.25	1.36	1.52
		α-Terpineol	**< 0.0001**	0.23	3.28	0.88	0.88	0.03	0.36	0.14	0.10
		β-Citronellol	**< 0.0001**	0.74	3.52	1.89	0.67	0.01	0.93	0.23	0.23
		Nerol	**< 0.0001**	0.33	16.44	11.84	3.87	0.33	5.23	1.72	1.29
		Geraniol	**< 0.0001**	3.59	53.51	30.96	10.86	2.07	5.89	3.20	1.23
		p-Cymene	**< 0.0001**	0.00	0.12	0.07	0.02	0.00	0.00	0.00	0.00

[1] s.d.is short for standard deviation. Bold values show significant correlation (Pearson, $\alpha = 0.05$).

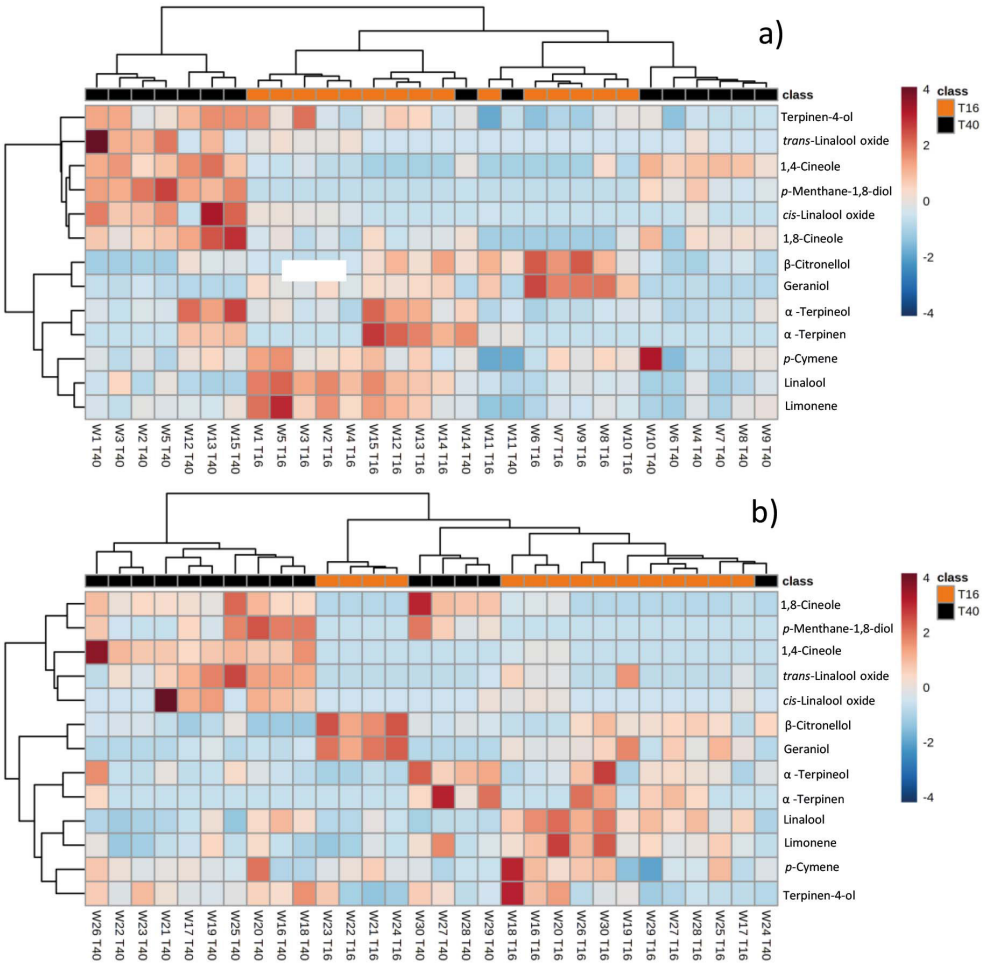

FIGURE 1. Heatmap of a) Corvina and b) Corvinone T16 and T40 wines.

with increased content of geraniol and β-citronellol, the other with increased levels of terpinen-4-ol, *p*-cymene, linalool, and in some wines also of α-terpineol and γ-terpinene. Upon ageing (T40 samples), these clusters were only in part preserved, so that of the wines initially present in the first cluster, only W21-23 were still relatively close, being characterised by moderate to high content of cineoles. Conversely, wines from the other T16 cluster were now in two distinct groups, one associated with increased content of α-terpineol, γ-terpinene, *p*-methane-1,8-diol, and 1,8-cineole (W27-30), the other with moderate to high levels of cineoles, terpene oxides, *p*-methane-1,8-diol.

Overall, these observations indicate that, while the terpene profile of aged Valpolicella wines was substantially different from that of the corresponding young wines, the transformations taking place during ageing were not following patterns clearly associated with the initial content of specific 'key' terpenes. Figure 2 summarises the main chemical reactions considered in this study. Free monoterpene alcohols act as potential precursors to a variety of other terpenes through acid-catalysed cyclisations, in particular leading to cyclic terpene alcohols that can, in turn, generate terpene diols and eventually bicyclic terpenes such as cineoles (Skouroumounis and Sefton, 2000; Slaghenaufi and Ugliano, 2018). One exception is the monoterpene alcohol β-citronellol (which contributed to characterising one of the clusters in Figure 1) which is less prone to acid rearrangements or hydration reactions due to the non-allylic structure of the related carbocation. Overall, the fact that monoterpene alcohols were mostly associated with young wines is therefore somewhat expected. At the same time, monoterpene alcohols, particularly geraniol, are also present in glycosidic forms, which can generate free terpenes in the acidic wine environment (Skouroumounis and Sefton, 2000). Likewise, many cyclic terpenes are also present in young wines, as they can originate directly from the grapes or relatively fast reactions occurring during winemaking.

To investigate the relationships between these various groups of terpenes, a correlation study was undertaken (Table 3). Correlation coefficients between free terpenes in T16 and T40 wines were generally low, with the exclusion of linalool and limonene, which were well correlated ($R^2 > 0.8$) with *p*-menthane-1,8-diol. This apparent lack of

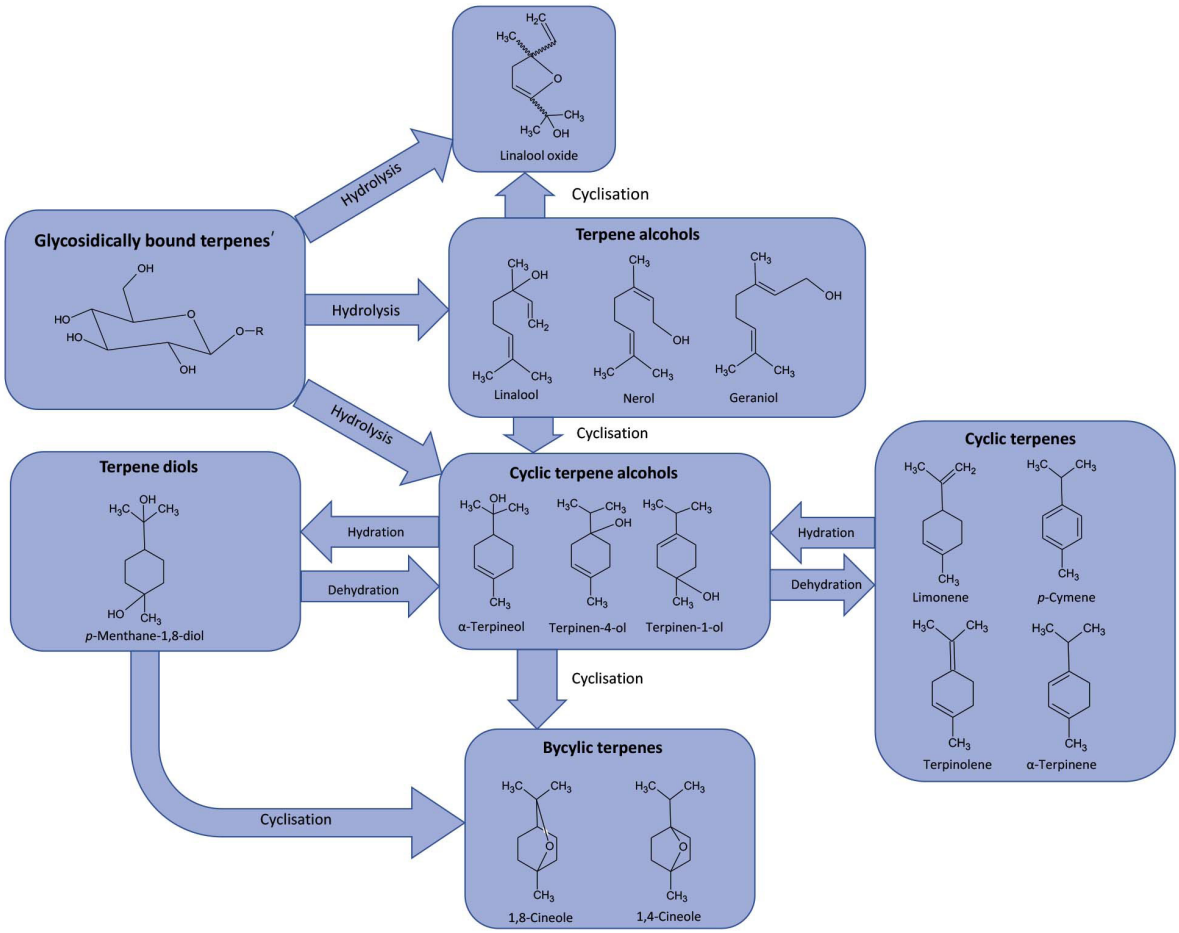

FIGURE 2. Main terpene reactions considered.

any clear relationship between the terpene profile of young wines and that of aged wines reflects to some extent the complexity of terpene reactions in wine hydroalcoholic acidic environment as well as suggesting the existence of modulating factors influencing the actual rate of formation/degradation of the various terpenes. The main terpenes of young wines, in particular linear monoterpene alcohols, are subject to cyclisation and hydration reactions at wine pH, so that their disappearance is somewhat expected, although other terpenes can be generated from them. Some ageing-related terpenes, including the cyclic monoterpene alcohol α-terpineol, as well as the other cyclic terpenes derived from the degradation of monoterpene alcohols, are intermediates in the process of formation of other cyclic and bicyclic terpenes and, therefore, they might not accumulate in a way that reflects the original content of their possible precursors (Slaghenaufi and Ugliano, 2018). More surprising was the observation that glycosidically-bound forms of the various terpenes detected, which were predominantly of linalool and geraniol, also did not appear to account for any of the terpenes observed in aged wines, with correlation coefficients that were even lower than those of the free compounds. Acid hydrolysis of monoterpene glycosides is considered a significant route to terpene formation at wine pH (Williams *et al*., 1982), so a certain degree of correlation between glycosylated terpenes of young wines and free terpenes of aged wines would have been expected. However, the formation of terpenes such as cineoles from monoterpene glycosides occurs at extremely harsh pH, while it might be less favoured at wine pH (Williams *et al*., 1982). Additionally, Skouroumounis and Sefton (2000) observed much higher reaction yields for hydrolytic conversion of free geraniol into linalool, α-terpineol and other cyclic terpenes compared to geraniol glucoside, indicating that, at wine pH, the terpene profile of aged wines could be more dependent of free terpene of young wines.

One set of generally good and significant correlations that were observed across the entire dataset was the one involving linalool, *p*-menthane-1,8-diol and 1,8-cineole. This was interesting as cineoles have been recently indicated as sensorially relevant to the aroma of aged red wines, where they can contribute to minty, balsamic and hay characters (Antalick *et al*., 2015; Black *et al*., 2015; Poitou *et al.,* 2017; Slaghenaufi and Ugliano, 2018). While some authors have reported a possible exogenous origin for cineoles via airborne migration (Hervé *et al.,* 2003), their occurrence in aged wines appears to be mostly related to the degradation of other terpenes during ageing (Fariña *et al*., 2015, Slaghenaufi and Ugliano, 2018). According to Williams *et al* (1982), cineoles can arise

TABLE 3. Correlation (R^2) between terpenes in aged wines and their free and glycosidically-bound forms in young wines in Corvina and Corvinone wines together.

		Free Terpenes T40 (µg/L)												
	Variables	*p*-Menthane-1,8-diol	*cis*-Linalool oxide	*trans*-Linalool oxide	1,4-Cineole	Limonene	1,8-Cineole	*p*-Cymene	Linalool	Terpinen-4-ol	α-Terpineol	β-Citronellol	Nerol	Geraniol
		R^2	R^2	R^2	R^2	R^2	R^2	R^2	R^2	R^2	R^2	R^2	R^2	R^2
Free terpenes T16	*p*-Menthane-1,8-diol	**0.343**	**0.333**	**0.282**	0.034	**0.067**	0.025	0.001	**0.096**	0.017	0.002	**0.089**	0.004	0.027
	cis-Linalool oxide	**0.246**	**0.479**	**0.339**	0.046	**0.075**	< 0.0001	0.004	**0.437**	0.028	**0.091**	**0.396**	0.030	**0.609**
	trans-Linalool oxide	**0.462**	**0.598**	**0.443**	**0.113**	**0.107**	0.015	0.000	**0.465**	**0.102**	0.057	**0.434**	0.004	**0.247**
	1,4-Cineole	**0.089**	**0.132**	**0.107**	**0.170**	**0.108**	0.018	**0.074**	**0.112**	**0.071**	0.034	**0.072**	0.014	0.023
	Limonene	**0.870**	**0.335**	**0.264**	**0.398**	**0.489**	**0.475**	**0.130**	**0.175**	**0.460**	**0.188**	**0.078**	0.002	0.042
	1,8-Cineole	**0.565**	**0.149**	**0.116**	**0.170**	**0.226**	**0.288**	**0.134**	**0.138**	**0.209**	0.037	**0.146**	0.000	0.062
	p-Cymene	**0.479**	**0.128**	**0.110**	**0.538**	**0.251**	**0.376**	**0.191**	**0.102**	**0.365**	0.053	**0.090**	0.058	0.002
	Linalool	**0.822**	**0.312**	**0.250**	**0.317**	**0.470**	**0.449**	**0.096**	**0.281**	**0.500**	**0.211**	**0.087**	0.001	**0.074**
	Terpinen-4-ol	**0.435**	**0.232**	**0.141**	**0.401**	**0.121**	**0.243**	**0.094**	**0.302**	**0.620**	0.045	**0.122**	0.001	**0.095**
	α-Terpineol	**0.320**	0.001	0.004	**0.199**	**0.302**	**0.766**	**0.141**	0.013	**0.493**	**0.880**	**0.089**	0.024	0.057
	β-Citronellol	**0.266**	**0.352**	**0.214**	0.021	0.018	0.060	0.000	**0.183**	**0.103**	0.003	**0.290**	**0.075**	**0.327**
	Nerol	0.059	0.025	0.008	0.003	0.004	0.040	0.003	0.056	**0.115**	0.010	0.001	0.014	0.052
	Geraniol	**0.145**	**0.120**	**0.067**	0.000	0.006	0.052	0.015	**0.128**	**0.087**	0.045	0.044	0.031	0.046
Glycosidically bound terpenes T16	*cis*-Linalool oxide	**0.124**	**0.344**	**0.148**	**0.072**	0.012	0.000	0.008	0.051	0.006	**0.108**	**0.087**	**0.075**	0.003
	trans-Linalool oxide	**0.146**	**0.345**	**0.141**	**0.091**	0.022	0.002	0.018	0.059	0.007	**0.110**	**0.098**	**0.073**	0.003
	Linalool	0.055	0.000	**0.093**	**0.094**	**0.104**	0.000	0.029	**0.192**	0.030	< 0.0001	**0.162**	0.031	**0.101**
	α-Terpineol	**0.159**	**0.065**	< 0.0001	**0.077**	0.025	**0.176**	0.028	0.003	**0.136**	**0.070**	0.051	0.047	0.021
	β-Citronellol	**0.122**	0.042	0.000	**0.146**	0.016	**0.118**	**0.159**	< 0.0001	**0.074**	0.000	0.007	**0.068**	0.039
	Nerol	**0.141**	0.008	0.025	**0.095**	0.012	0.057	0.025	0.008	**0.144**	0.056	0.057	**0.108**	0.048
	Geraniol	**0.198**	0.034	0.001	**0.107**	0.062	**0.076**	**0.072**	0.031	**0.091**	0.007	0.008	**0.091**	0.018
	p-Cymene	**0.100**	0.057	**0.093**	**0.214**	**0.145**	0.012	**0.152**	**0.075**	0.006	0.064	**0.184**	0.039	0.004

Bold values show significant correlation (Pearson, $\alpha = 0.05$).

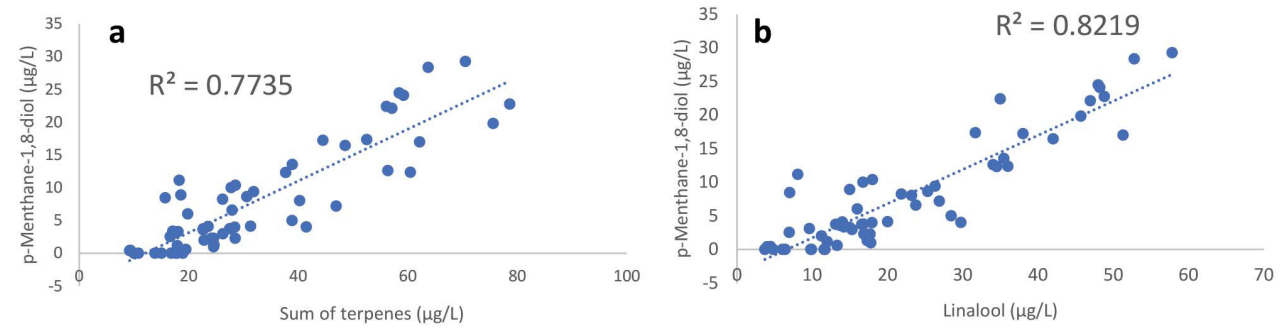

FIGURE 3. Correlation between a) the sum of linalool, geraniol, nerol and α-terpineol linalool and b) linalool in T16 wines with p-menthane-1,8-diol in T40 wines.

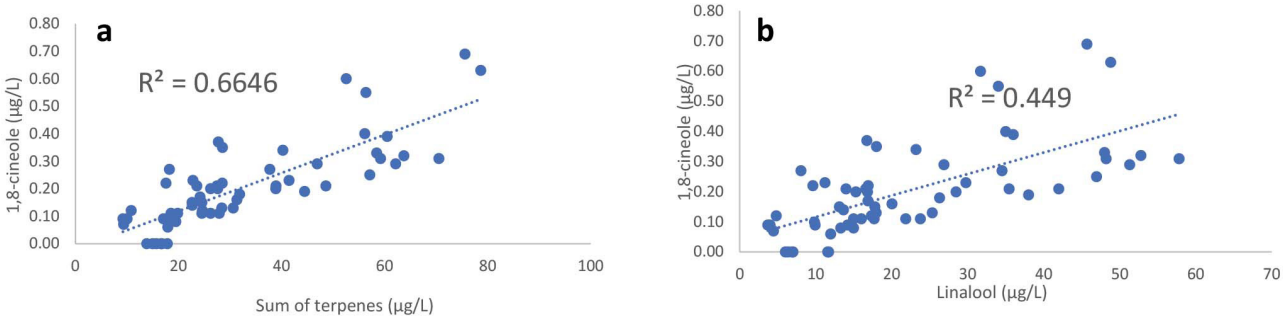

FIGURE 4. Correlation between a) the sum of linalool, geraniol, nerol and α-terpineol linalool and b) linalool in T16 wines with 1,8-cineole in T40 wines.

from harsh acid hydrolysis of glycosylated terpenes. Slaghenaufi and Ugliano (2018) proposed at wine pH a formation pathway involving free forms of the monoterpene alcohols linalool, geraniol, nerol and α-terpineol as precursors, and in which *p*-menthane-1,8-diol is an intermediate in the formation of 1,8-cineole but also 1,4-cineole. We found that *p*-menthane-1,8-diol in aged samples was very well correlated with the sum of linalool, geraniol, nerol and α-terpineol in young wines (R^2 = 0.7735) and, in particular, with linalool (R^2 = 0.8219), the most abundant monoterpene alcohol in young wines (Figure 3a,b). Conversely, the content of glycosylated terpenes in young wines was not associated with increased *p*-menthane-1,8-diol formation. As for cineoles, the sum of free linalool, geraniol, nerol and α-terpineol in young wines was reasonably well correlated (R^2 = 0.6646) with 1,8-cineole in aged wines, whereas the correlation was less strong (R^2 = 0.449) for linalool alone (Figure 4a,b). Considering that the sample set contained wines of three different vintages, which might differ for other matrix-related factors other than terpene profiles, additional insights were obtained when correlations were assessed within individual vintages. Correlations between 1,8-cineole and either the sum of free linalool, geraniol, nerol and α-terpineol or linalool alone were good within each vintage, with vintages 2018 and 2019 showing a similar slope while vintage 2017 showed a lower slope, and thus a lower yield of 1,8-cineole from the two possible precursors (Figure 5a and 5b). Further evaluation of the possible drivers of such differences in the reaction yields indicated that pH was a major factor differentiating the three vintages, with 2017 showing higher and less dispersed values than the other two vintages, which tended to be on the lower end of the pH values. Accordingly, across the three vintages, the wines could be effectively divided into two groups showing different yields, with a pH value of 3 appearing to be the threshold between lower and higher yields (Figure 5c,d). Overall, a significant negative correlation ($p < 0.0001$, Pearson) between pH and the yield of 1,8-cineole from either free linalool or the sum of free linalool, geraniol, nerol and α-terpineol was observed (Table 4), so that a decrease in compound yields was observed at increased pH. Regarding 1,4-cineole in aged wines, correlations with either the sum of free monoterpenes as well as of linalool in young wines were not as good as those observed for 1,8-cineole (R^2 = 0.368 and 0.317, respectively). Segmentation of wine samples by either vintage or pH did result in higher correlation coefficients (Figure 6a–d), indicating a certain modulating effect of pH in this case too. However, especially in the low pH samples, several cases were observed in which wines with relatively high total monoterpene or linalool content did not give any 1,4-cineol, as well as one case in which 1,4-cineole levels were much lower than expected. Therefore, other relevant modulation factors are likely to exist, and the relationship between reaction yield and pH appeared less strong for this molecule (Table 4). The formation of 1,4-cineole from monoterpene alcohols requires more steps than that of

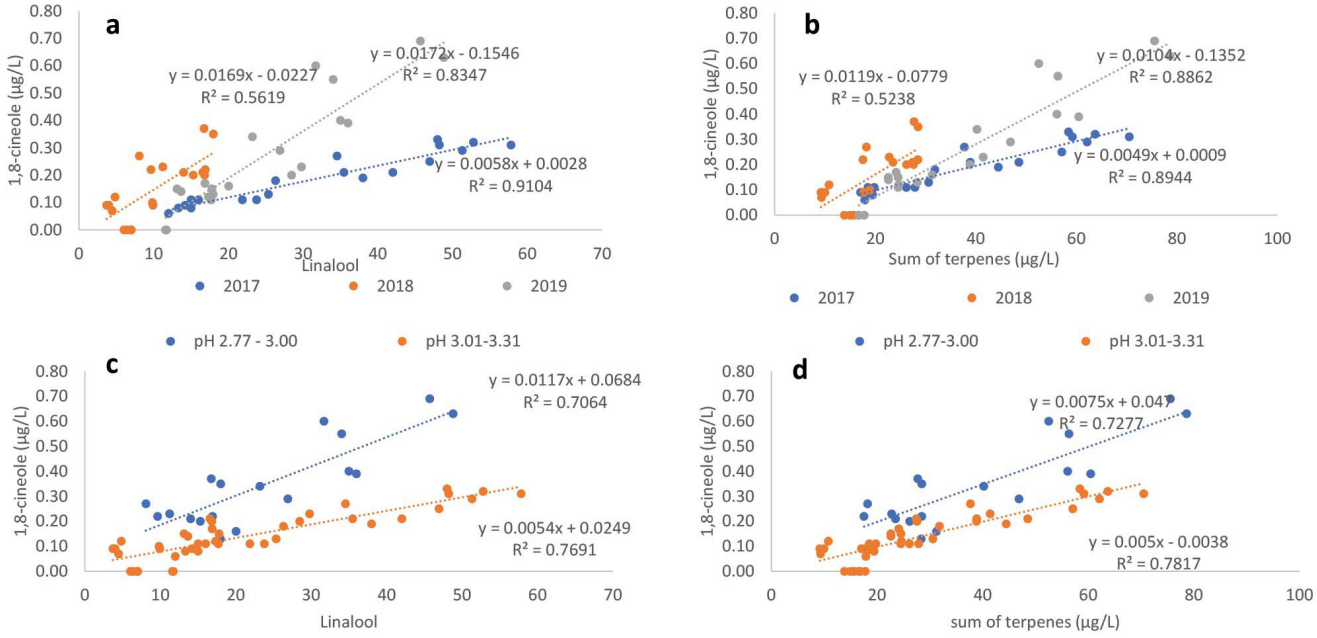

FIGURE 5. Correlation between a) linalool and b) the sum of linalool, geraniol, nerol and α-terpineol in T16 wines with 1,8-cineole in T40 wines considering different vintages. Correlation between c) linalool and d) the sum of linalool, geraniol, nerol and α-terpineol in T16 wines with 1,8-cineole in T40 wines considering separately wines with pH greater or lower than 3.00.

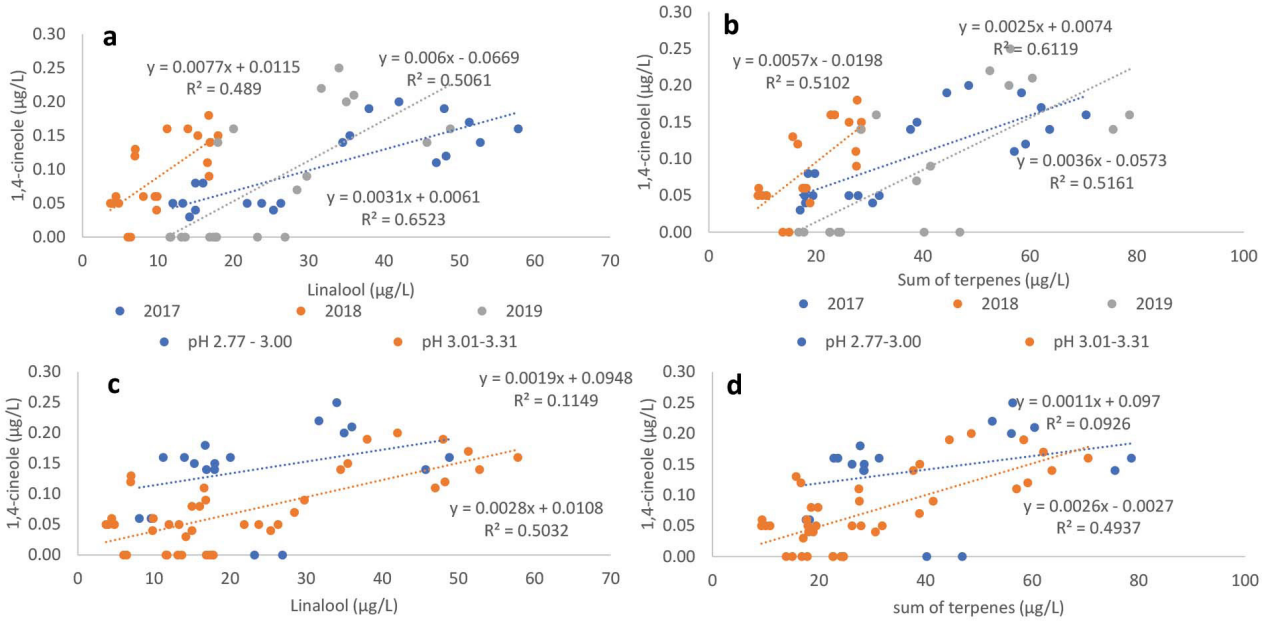

FIGURE 6. Correlation between a) linalool and b) the sum of linalool, geraniol, nerol and α-terpineol in T16 wines with 1,4-cineole in T40 wines considering different vintages. Correlation between c) linalool and d) the sum of linalool, geraniol, nerol and α-terpineol in T16 wines with 1,4-cineole in T40 wines considering separately wines with pH greater or lower than 3.00.

1,8-cineole (Slaghenaufi and Ugliano, 2018), so that less robust correlations are somewhat to be expected. Among the additional proposed intermediates leading to 1,4-cineole (Figure 2), the content of the cyclic terpene terpinen-4-ol in aged wines was well correlated with the sum of monoterpene alcohols of young wine (Figure 7), supporting the hypothesis that monoterpene content of young wines contributes to the 1,4-cineole formation during ageing.

Overall, these data indicate that, of the different forms of terpenes present in Valpolicella young red wines, free monoterpene alcohols account mostly for the formation of potent terpene odorants such as cineoles during ageing,

TABLE 4. Correlation between pH and the yield of 1,4- and 1,8-cineole from free linalool and the sum of free terpenes (linalool, geraniol, nerol and α-terpineol).

	Pearson correlation coefficient	p-value
1,8-Cineole/Linalool	-0.615	< 0.0001
1,8-Cineole/sum of terpenes	-0.677	< 0.0001
1,4-Cineole/Linalool	-0.285	0.027
1,4-Cineole/sum of terpenes	-0.318	0.013

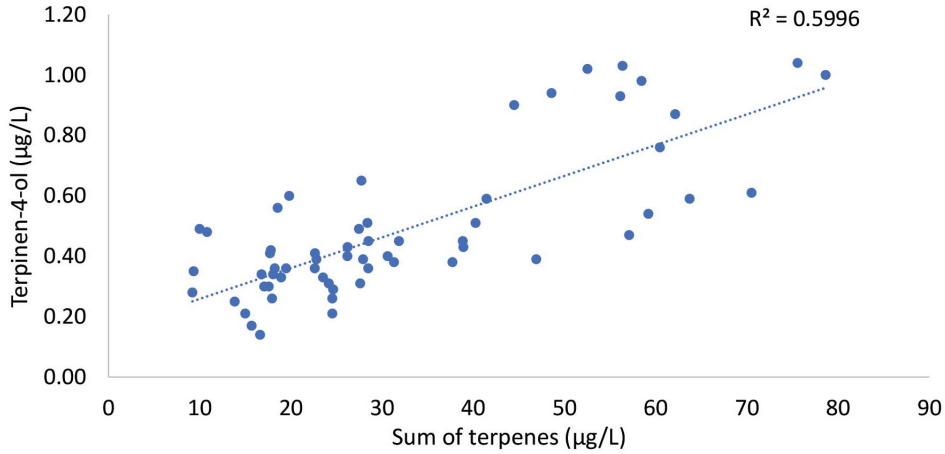

FIGURE 7. Correlation between the sum of linalool, geraniol, nerol and α-terpineol in T16 wines and terpinen-4-ol in T40 wines.

whereas glycosylated monoterpene alcohols appear to play a minor role. pH is a major modulator of the actual yield of cineoles formation from monoterpene alcohols, so that, depending on pH, young wines that are relatively rich in monoterpenes might not give, upon ageing, high cineoles content, and vice-versa. The influence of pH appears to be related to the last steps of cineoles formation, whereas conversion of the different monoterpene alcohols into the cyclic intermediate *p*-menthane-1,8-diol was not pH-dependent.

CONCLUSIONS

Ageing can radically change the terpene profile of a wine. The terpene profile of young Valpolicella red wines appeared characterised by a relatively high content of different monoterpene alcohols, in particular in free forms. These free monoterpene alcohols account mostly for the formation of potent terpene odorants such as cineoles during ageing, whereas glycosylated monoterpene alcohols appear to play a minor role. pH is a major modulator of the actual yield of cineoles formation from monoterpene alcohols, so that, depending on pH, young wines that are rich in monoterpenes might not give upon ageing high cineoles content, and vice-versa. The influence of pH appears to be related to the last steps of cineoles formation, whereas conversion of the different monoterpene alcohols into the cyclic intermediate *p*-menthane-1,8-diol was not pH-dependent. The results of this study provide novel insights into the mechanism associated with aroma development during ageing, highlighting the significant contribution of the pool of free terpenes in young wines. Further studies should be conducted on model solutions containing different potential precursors and possibly other major matrix components (e.g. phenolics) to validate this observation and further classify red wine aroma ageing potential in other varieties. The observation that the transformation of young wines terpenes into ageing-related ones can be significantly impacted by pH indicate opportunities for fine-tuning aroma development in relation to expected wine shelf-life.

ACKNOWLEDGEMENTS

The authors thank the winery Fratelli Tedeschi for financial support and for providing the grapes employed.

REFERENCES

Accordini, D. (2013). Amarone. In F. Mencarelli, & P. Tonutti, *Sweet, reinforced and fortified wine: Grape biochemistry, technology and vinification* (p. 189-203). Wiley and Blackwell Ltd. https://doi.org/10.1002/9781118569184.ch13

Antalick, G., Perello, M.-C., & de Revel, G. (2014). Esters in Wines: New Insight through the Establishment of a Database of French Wines. *American Journal of Enology and Viticulture*, 65, 293-304. https://doi.org/10.5344/ajev.2014.13133

Antalick, G., Tempère, S., Katja , Š., Blackman, J., Deloire, A., de Revel, G., & Schmidtke, L. (2015). Investigation and Sensory Characterisation of 1,4-Cineole: A Potential Aromatic Marker of Australian Cabernet Sauvignon Wine. *Journal of Agricultural and Food Chemistry*, 63(41), 9103-9111. https://doi.org/10.1021/acs.jafc.5b03847

Bellincontro, A., Matarese, F., D'Onofrio, C., Accordini, D., Tosi, E., & Mencarelli, F. (2016). Management of postharvest grape withering to optimise the aroma of the final wine: A case study on Amarone. *Food Chemistry*, 213, 378-387. https://doi.org/10.1016/j.foodchem.2016.06.098

Black, C., Parker, M., Siebert, T., Capone, D., & Francis, I. (2015). Terpenoids and their role in wine flavour: recent advances. *Australian Journal of Grape and Wine Research*, 21, 582–600. https://doi.org/10.1111/ajgw.12186

D'Agata, I. (2019). *Italy's Native Wine Grape Terroirs* (p. 70-81). Berkeley: University of California Press. https://doi.org/10.1525/9780520964778

Díaz-Maroto, M. C., Schneider, R., & Baumes, R. (2005). Formation Pathways of Ethyl Esters of Branched Short-Chain Fatty Acids during Wine Aging. *Journal of Agricultural and Food Chemistry*, 53(9), 3503–3509. https://doi.org/10.1021/jf048157o

Fariña, L., Villar, V., Ares, G., Carrau, F., Dellacassa, E., & Boido, E. (2015). Volatile composition and aroma profile of Uruguayan Tannat wines. *Food Research International*, 69, 244-155. https://doi.org/10.1016/j.foodres.2014.12.029

Fariña, L., Boido, E, Carrau, F., Versini, G., & Dellacassa, E. (2005). Terpene Compounds as Possible Precursors of 1,8-Cineole in Red Grapes and Wines. *Journal of Agricultural and Food Chemistry*, 53(5), 1633–1636. https://doi.org/10.1021/jf040332d

Hervé, E., Price, S., & Burns, G. (2003). *Eucalyptol in wines showing a "eucalyptus" aroma. Proceedings of VIIeme Symposium International d'Oenologie* (p. 598–600.). A. Lonvaud, G. de Revel, and P. Darriet.

Luzzini, G. (2021a). *Aroma chemical signatures of wine geographical origin in relationship to grape variety, sensory characteristics, and technological factors. A case study on Valpolicella red wines*. Università degli studi di Verona: PhD thesis.

Luzzini, G., Slaghenaufi, D., Pasetto, F., & Ugliano, M. (2021b). Influence of grape composition and origin, yeast strain and spontaneous fermentation on aroma profile of Corvina and Corvinone wines. *Lebensmittel-Wissenschaft & Technologie*, 143, 1-13. https://doi.org/10.1016/j.lwt.2021.111120

Luzzini, G., Slaghenaufi, D., & Ugliano, M. (2021c). Volatile Compounds in Monovarietal Wines of Two Amarone Della Valpolicella Terroirs: Chemical and Sensory Impact of Grape Variety and Origin, Yeast Strain and Spontaneous Fermentation. *Foods*, 10(10), 2474-2493. https://doi.org/10.3390/foods10102474

McKay, M., Buglass, A. J., & Lee, C. G. (2010). Wine. In A. Buglas, *Handbook of Alcoholic Beverages: Technical, Analytical and Nutritional Aspects* (p. 266–382.). Chichester: John Wiley & Sons, Ltd. https://doi.org/10.1002/9780470976524.ch12

MIPAAF (2019). Ministero delle Politiche Agricole Alimentari e Forestali. Dipartimento delle politiche competitive, della qualità agroalimentare, ippiche e della pesca. Disciplinare di produzione dei vini a Denominazione di Origine Controllata e Garantita. "Amarone della Valpolicella". G. U. n.190 del 14.08.2019.

Moio, L. (2016). *L'amarena e il pepe della Corvina. In L. Moio, Il Respiro del Vino* (p. 459.). Milano: Mondadori.

Poitou, X., Thibon, C., & Darriet, P. (2017). 1,8-Cineole in French red wines: evidence for a contribution related to its various origins. *Journal of Agricoultural and Food Chemistry*, 65(2), 383–393. https://doi.org/10.1021/acs.jafc.6b03042

Rapp, A., & Mandery, H. (1986). Wine aroma. Experientia, 42, 873-884. https://doi.org/10.1007/BF01941764

Skouroumounis, G. K., & Sefton, M. A. (2000). Acid-catalysed Hydrolysis of Alcohols and Their β-D-glucopyranosides. *Journal of Agricultural and Food Chemistry*, 48(6), 2033-2039. https://doi.org/10.1021/jf9904970

Slaghenaufi, D., Vanzo, L., Luzzini, G., Arapitsas, P., Marangon, M., Curioni, A., Mattivi, F., Piombino, P., Moio, L., Versari, A., Ricci, A., Rio Segade, S., Rolle, L., & Ugliano, M. (2022). Monoterpenoids and norisoprenoids in Italian red wines. *OENO One*, 56(3), 185–193. https://doi.org/10.20870/oeno-one.2022.56.3.5387

Slaghenaufi, D., & Ugliano, M. (2018). Norisoprenoids, Sesquiterpenes and Terpenoids Content of Valpolicella Wines During Aging: Investigating Aroma Potential in Relationship to Evolution of Tobacco and Balsamic Aroma in Aged Wine. *Frontiers in Chemistry*, 6, 1-13. https://doi.org/10.3389/fchem.2018.00066

Slaghenaufi, D., Guardini, S., Tedeschi, R., & Ugliano, M. (2019). Volatile terpenoids, norisoprenoids and benzenoids as markers of fine scale vineyard segmentation for Corvina grapes and wines. *Food Research International*, 125, 1-10. https://doi.org/10.1016/j.foodres.2019.108507

Slaghenaufi, D., Peruch, E., De Cosmi, M., Nouvelet, L., & Ugliano, M. (2021). Volatile and phenolic composition of monovarietal red wines of Valpolicella appellations. *OENO One*, 55(1), 279-294. https://doi.org/10.20870/oeno-one.2021.55.1.3865

Tarasov, A., Giuliani, N., Dobrydnev, A., Schuessler, C., Volovenko, Y., Rauhut, D., & Jung, R. (2020). 1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN) Sensory Thresholds in Riesling Wine. *Foods*, 9, 606-618. https://doi.org/10.3390/foods9050606

Ugliano, M., Bartowsky, E. J., McCarthy, J., Moio, L., & Henschke, P. A. (2006) Hydrolysis and transformation of grape glycosidically bound volatile compounds during fermentation with three Saccharomyces yeast strains. *Journal of Agricultural and Food Chemistry*, 54(17), 6322-6331. https://doi.org/10.1021/jf0607718

Ugliano, M. (2013). Oxygen Contribution to Wine Aroma Evolution during Bottle Aging. *Journal of Agricultural and Food Chemistry*, 61(26), 6125–6136. https://doi.org/10.1021/jf400810v

Wedler, H. B., Pemberton, R. P., & Tantillo, D. J. (2015). Carbocations and the complex flavor and bouquet of wine: mechanistic aspects of terpene biosynthesis in wine grapes. *Molecules*, 20, 10781–10792. https://doi.org/10.3390/molecules200610781

Williams, P. J., Strauss, C. R., Wilson, B., & Massy-Westropp, R. A. (1982). Studies on the hydrolysis of *Vitis vinifera* monoterpene precursor compounds and model monoterpene β-D-glucosides rationalising the monoterpene composition of grapes. *Journal of Agricultural and Food Chemistry*, 30(6), 1219-1223. https://doi.org/10.1021/jf00114a054.