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A unique mixture of monoterpenes and volatile phenols characterises Zelen wine's aromatic profile

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ABSTRACT

This study aimed to characterise the aromatic uniqueness of Zelen (*Vitis vinifera* L.) wines, an autochthonous variety from the Vipava Valley located in Western Slovenia, through chemical and sensory assessment. Seventy aromatic compounds, including varietal thiols, esters, C6-alcohols, volatile phenols, terpenoids, and norisoprenoids, were analysed by HS-SPME-GC-MS in two surveys comparing Zelen wines with four other varieties grown in Vipava Valley. The olfactory space of Zelen wines was defined by comparing their aromatic profiles with those of Pinela wines in a sorting task and by the sniffing of aromatic fractions obtained by HPLC fractionation. Zelen wines were characterised by dried herbs and spicy notes such as thyme, rosemary, and basil in comparison to Pinela wines. The chemical profile of Zelen wines was dominated by the presence of an original mixture of monoterpenes, including terpinene isomers, limonene, *p*-cymene, terpinolene, linalool, and α-terpineol, as well as volatile phenols, 4-vinylguaiacol, and methyl salicylate. The obtained concentrations of 4-vinylguaiacol and methyl salicylate were at levels close to or above the reported olfactory threshold, thus inferring a potential contribution of these compounds to the spicy aromatic components of Zelen wines. Two aromatic fractions reminiscent of herbal notes specific to Zelen wines–isolated by HPLC semi-preparative fractionation and further analysed by HS-SPME-GC-MS–revealed the presence of an original mixture of hydrocarbon monoterpenes, including the compounds that were quantitatively measured, and others, such as β-myrcene, β-phellandrene, E-β-ocimene, Z-β-ocimene, and two 2,4,6-octatriene-2,6-dimethyl isomers. Semi-quantitative measurements showed that this new group of monoterpenes was also present at higher levels in Zelen than in Pinela, Malvasia Istriana, Chardonnay, and Sauvignon blanc wines.

KEYWORDS: autochthonous variety, Zelen, typicality, aroma, methyl salicylate, 4-vinylguaiacol, terpenes

INTRODUCTION

Market interest in autochthonous varieties, particularly from less-known wine regions, has significantly increased in the past decade. The share of wine consumers eager to discover new regions and varieties with distinctive sensory profiles has increased steadily (Robinson, 2020). The presence of autochthonous grape varieties in wines is generally seen as positive by consumers, who value the link between their typicality and their ties with their territory (Boncinelli *et al*., 2016). Wine typicality, which is related to its origin and variety, has been recognised as a key dimension of wine quality (Charters & Pettigrew, 2007; Darriet *et al*., 2013). Wine typicality is not only relevant to the economic sustainability of wine producers (Casini *et al*., 2015) but is also crucial in terms of rural development (Boncinelli *et al*., 2016). While the concept of typicality has been investigated for several decades (Rosch & Mervies, 1975; Loken & Ward, 1990), studies investigating wine varietal and regional typicalities have mainly focused on wine aroma, most of which were performed from 2000 onwards (Souza Gonzaga *et al*., 2021). Many studies have aimed to characterise the aromatic typicality of important international varieties, such as Chardonnay (Balester *et al*., 2005), Sauvignon blanc (Tominaga *et al*., 1998, Paar *et al*., 2007), Pinot noir (Tomasino *et al*., 2013), Shiraz (Pearson *et al*., 2020) or Cabernet-Sauvignon (Antalick *et al*., 2015a) through sensory and chemical analyses. Similar studies have recently been performed on less-known regional varieties (Slaghenaufi *et al*., 2021; Nanou *et al*., 2020; Laureati *et al*., 2023). At first sight, the knowledge gained from such studies seems to be relevant only for targeted regions. Nevertheless, some of these regional varieties have such a unique profile that the outputs from these studies can be insightful for the whole field of wine sciences. For instance, Guedes de Pinho *et al.* (2007) were the first to highlight the important contribution of terpenes to the aromatic profile of non-aromatic red grape varieties. Several studies have demonstrated that this group of aromatic compounds affects the aromatic profiles of a wide range of red wines (Wood *et al*., 2008; Antalick *et al*., 2015; Slaghenaufi *et al*., 2019). More recently, the characterisation of the Lugana and Verdicchio aromatic signatures highlighted the influence of methyl salicylate on white wine aroma (Carlin *et al*., 2019; Slaghenaufi *et al*., 2021). In the past two years, this compound has suddenly gained interest in the wine science community, with several works published on this topic (Poitou *et al*., 2021; Pelonnier-Magimel *et al*., 2022; Slaghenaufi *et al*., 2022; Piergiovanni *et al*., 2023).

Zelen (*Vitis vinifera* L.) is a white grape variety only grown in the Vipava Valley wine region in western Slovenia, close to the Italian border. It was already described as a "noble variety" by an important local agronomist in the middle of the 19th century (Škvarč *et al*., 2006). The variety was nearly extinct just after the Second World War, with less than 2 ha planted in the Vipava Valley wine region because of its low yield, while producers were more interested in volume than quality. However, Zelen underwent a revival 20 years ago when several producers started to produce higher-quality wines by better expressing their uniqueness in classic and macerated white wine styles. Currently, Zelen is planted over 85 ha and has become one of the trendiest regional varieties in Slovenia, particularly because of its unique aromatic profile. However, besides ampelographic data, knowledge of Zelen grape and wine characteristics is limited. Štajner *et al*. (2008) showed that the Zelen variety is in fact a heterogeneous group consisting of several genotypes. Zelen wines tend to have moderate levels of alcohol and display in the glass some shades of green from where its name comes from as "zelen" means "green" in Slovenian. Bavčar *et al*. (2016) compared the chemical and sensory profile of Zelen wines with two other regional varieties made with different alternative skin contact procedures. The results suggested that Zelen displayed a more intense floral and phenolic-spicy aroma, likely due to the higher content of terpenols and 4-vinylguaiacol, respectively. However, it is difficult to draw a general conclusion on the aromatic typicality of Zelen wine from this work, because these wines were made under micro-vinification conditions from one vineyard and one vintage. In the sensory description published in wine media, singular aromas of Zelen wines are often described as dried herbs, fruity, and floral notes. This study aimed to scientifically characterise the aromatic signature of Zelen wines through chemical and sensory analyses. Chemical surveys of different single-variety white wines from the Vipava Valley wine region were performed to identify potential markers of Zelen wine typicality. To evaluate whether Zelen possessed a distinct olfactory space, a sorting task analysis was carried out. The potential influence of the chemical markers identified in the survey on the Zelen wine aroma was also explored by sensory analysis.

MATERIALS AND METHODS

1. Wines

Wines were sourced from specialised wine shops and directly from the producers. Two different sets of wines were subjected to chemical analysis. The first survey was performed in November 2021, focusing on varietal thiols that were analysed with a highly time-consuming quantitative method. For this reason, the first survey was limited to twenty-six wines from Vipava Valley (80 %) and other Slovenian wine regions (20 %). Esters, C6-alcohols, volatile phenols, terpenoids, and norisoprenoids were analysed in a second survey carried out in October 2022. In this case, the wines were all from Vipava Valley which is known to have a sub-Mediterranean climate with continental influence in the upper valley where most of the Zelen vineyards are located. Wines were sourced from 2018 to 2021 vintages characterised by average Huglin and cool night indices of 2466 and 12.95, respectively. These values corresponded to a warm climate with cool nights during these vintages (Tonietto & Carbonneau, 2004). Average seasonal precipitation (April-September) was 638 mm during the same period. Climatic data was sourced from a national weather station located in Slap (upper Vipava Valley). Zelen vineyards are planted on the terraced slopes and flat lands of Vipava Valley on sedimentary soils such as flysch and sandstones with variable proportions of clay. Selected wines were made according to diverse winemaking approaches and reflected the current market for all the varieties. For that reason, a higher proportion of one- and two-year-old wines was selected. Overall, the vintage selection was balanced as much as possible between varieties to limit the vintage effect in the wine chemical and sensory comparisons. The compositions of both sets of wines with their corresponding vintages are listed in Table 1.

TABLE 1. Number of wines and vintage per variety analysed in surveys 1 and 2.

	Survey 1			Survey 2			
	2018	2019	2020	2018	2019	2020	2021
Zelen	2	3	5	0	5	10	10
Pinela	1	2	2	1	1	7	13
Malvasia Istriana	1	4	0	1	1	3	2
Chardonnay	1	2	0	2	1	0	1
Sauvignon blanc	1	2	0	2	0	1	4

2. Chemical analysis

2.1. Varietal thiols

Three volatile thiols, namely 4-methyl-4-sulfanylpentan-2-one (4-MSP), 3-sulfanylhexanol (3-SH), and 3-sulfanylhexyl acetate (3-SHA), were determined in wines using previously published methods for sample preparation (Dubourdieu *et al*., 2006), with some modifications as described by Šuklje and Čuš (2021). Samples were thereafter analysed using a gas chromatograph GC 2030 Shimatzu (Kyoto, Japan) coupled to a triple quad mass detector (MS/MS) TQ8050NX Shimatzu (Kyoto, Japan), equipped with HP-INNOWAX, 60 m × 0.25 mm; film thickness 0.25 µm Agilent J&W GC column (Agilent Technologies, Santa Clara, USA). Helium gas was used as the carrier gas at a constant flow rate of 1 mL/min. The oven temperature was set as follows: 45 °C for 1 min, increased at a rate of 5 °C/min to 90 °C, increased at a rate of 4 °C/min to 174 °C, held for 2 min, increased at a rate of 20 min to 250 °C, and held for 10 min. The transitions, collision energies, and method validation parameters for the selected compounds are presented in the supplementary material (Table S1).

2.2. Esters, C6-alcohols, and volatile phenols

A previously developed headspace solid-phase microextraction gas chromatography-mass spectrometry (HS-SPME-GC-MS) method for analysing esters, C6 alcohols, and volatile phenols (VPs) allows the quantification of approximately 30 odorants (Martelanc *et al*., 2024).

2.3. Terpenes and norisoprenoids

Quantitative analyses of 22 monoterpenes, two sesquiterpenes, and four norisoprenoids in wines were performed as previously described (Martelanc *et al*., 2024). The concentration of eight additional monoterpenes was semi-quantitatively assessed using octan-2-ol as an internal standard, as described by Martelanc *et al*. (2024). β-myrcene; β-phellandrene; E-β-ocimene; Z-β-ocimene; two 2,4,6-octatriene-2,6-dimethyl isomers; habanene and cosmene. These compounds were identified in the Zelen wines in scan mode with their mass spectra and reported GC retention indices. The ions monitored in SIM for these compounds, m/z 93, 117, 119, and 121, were already used for other quantified monoterpenes.

2.4. Determination of hydroxycinnamic acids by HPLC-UV

An in-house developed and validated method for the determination of hydroxycinnamic acids was accomplished using an Agilent 1100 series HPLC system (Agilent Technologies©, Palo Alto, California, USA) equipped with a UV detector (detection at 284 nm). The samples were filtered using PTFE 0.45 µm disk filters and injected (10 µL) into a thermostated (30 °C) reversed-phase (RP) C18 Luna column (dimensions of 150 mm × 3.0 mm with a particle size of 3 µm) obtained from Phenomenex. The flow rate was set at 0.3 mL/min. The gradient elution and validation parameters are listed in Tables 2 and 3, respectively. Stock solutions of hydroxycinnamic acid were freshly prepared in a solution of ethanol/water = 1/1 (*v*/*v*). Calibration curve solutions were prepared by dissolving stock solutions with the same ethanol/water solution. All solutions were stored in a refrigerator at 4 °C. Control samples were prepared as well at the concentration levels being at the middle of the calibration ranges.

Absolute ethanol (99.8 %, HPLC grade) was obtained from Honeywell (Riedel-de Haën, Germany). Milli-Q water was obtained using a Purelab Option-Q system (ELGA LabWater, High Wycombe, UK). Sodium chloride (purity 99 %) was supplied from Chemlab (Zedelgem, Belgium). Acetonitrile (gradient grade, 99.9 %) and sulfuric acid (95–97 %) were purchased from Supelco (Germany). Caffeic acid (98.0 % HPLC), ferulic acid (99.9 %), and *p*-coumaric acid (98.0 % HPLC) were purchased from Sigma (Steinheim Germany), and caftaric acid (> 97 %) was bought from Sigma-Aldrich, St. Louis, USA.

TABLE 2. Gradient profile of the HPLC-UV method for determination of hydroxycinnamic acids.

Time (min)	Mobile phase A 5 mM H_2SO_4 (%)	Mobile phase B 95 % acetonitrile and 5 % mobile phase A (%)
0.00	94	6
7.00	94	6
22.00	25	75
22.10	0	100
25.00	0	100
25.10	94	6
37.00	94	6

TABLE 3. Validation parameters for the determination of hydroxycinnamic acids by HPLC-UV method.

Compounds	Retention time (min)	R^2	Repeatability[1] RSD (%)	Linearity[2] (y = kx + n)	LOD/LOQ[3] (mg/L)	Calibration range (mg/L)	Recovery[4] (%)
Caftaric acid	9.3	0.9998	1.2	y = 9.3x – 0.54	30/90	0.120 –120.0	94
Caffeic acid	14.9	0.9995	1.3	y = 17.3x – 1.2	20/60	0.060 – 12.0	100
p-Coumaric acid	19.6	0.9998	1.0	y = 20.8x + 2.4	20/60	0.060 – 12.0	94
Ferulic acid	21.9	0.9998	1.1	y = 11.6x + 0.3	20/60	0.060 – 12.0	93

[1] Repeatability was calculated as the relative standard deviation (RSD) by injecting one sample six times consecutively.

[2] Calibration curve was constructed using six calibration points.

[3] LOD and LOQ were defined as signal-to-noise ratio of 3/1 and 10/1, respectively.

[4] Recoveries were evaluated separately in triplicate by spiking a sample with 5 mg/L of each analyte, excluding caftaric acid spiked at 50 mg/L.

2.5. Wine extraction for HPLC-fractionation

The procedure for the extraction and concentration of aromatic compounds from wine samples was performed according to Lytra *et al*. (2012). Accordingly, a 750 mL wine sample was extracted successively using 80, 80, and 50 mL of dichloromethane, with a separatory funnel for 10 min. The organic phases were collected, blended, dried over sodium sulfate, and concentrated under nitrogen flow (100 mL/min) to obtain 1.25 mL of wine extract.

2.6. Semi-preparative HPLC on wine extracts and sensory evaluation

HPLC fractionation was performed on the same HPLC system as for the determination of hydroxycinnamic acids, using a fraction collector (FOXY® R1 FRACTION COLLECTOR, Teledyne Isco, Lioncoln, USA) at the end of the column elution. A concentrated solution of extracted aromas from wine samples was injected (200 µL) into a C18 Kinetex EVO (dimensions 250 × 4.6 mm) with a particle size of 5 µm) together with a precolumn (dimensions of 3 mm × 4.6 mm with a particle size of 5 µm) at a flow rate of 0.5 ¿mL/min using a gradient elution (water-A; ethanol-B) from 0 min to 5 min 100 % A, linearly programmed to 100 % B at minute 65. A total of 25 fractions (1 mL each) were collected. For sensory analysis, the level of ethanol in the fractions of interest was adjusted to 12 % ethanol (*v/v*) with ultrapure water and absolute ethanol (99.8 %, HPLC grade). Olfactory descriptions of the twenty-five aromatic fractions obtained by HPLC fractionation of one typical Zelen and one Pinela wine were performed by two panellists from the Wine Research Centre of the University of Nova Gorica who were trained to assess Zelen wine aromatic typicality. Fractions adjusted to 12 % ethanol (v/v) were collected into ISO glasses (ISO 3591:1977) before olfactory evaluation. Both experienced panellists discussed the odours of the different fractions and agreed on common descriptors. Due to the limited number of panellists, this sensory evaluation should not be considered as a sensory characterisation of Zelen aromatic fraction but rather as an analytical step that helped to highlight new markers of Zelen wine aromatic composition.

3. Sensory analysis

3.1. Sorting task

A descriptive sorting task was used to characterise the aromatic differences between five Zelen and five Pinela wines originating from the Vipava Valley region. In each group, two samples were from 2022 and three samples were from 2021 vintage. The sorting task was performed by orthonasal evaluation following the method described by Šuklje *et al*. (2015). The panellists were instructed to form between two and nine groups based on their aroma similarities. The 10 panellists who performed the sorting task were research staff at the Wine Research Centre, University of Nova Gorica, and wine professionals from Vipava Valley selected based on availability and interest. The ages of the panellists ranged from 30 to 55 years, and the panel comprised 60 % females. Thirty millilitres of wine samples were served at room temperature into ISO glasses (ISO 3591:1977) coded with random three-digit numbers. Subsequently, they were asked to indicate a minimum of four descriptors for each group from a list of 16 descriptors from fruity (citrus, peach/apricot, apple/pear, pineapple, banana, passion/tropical fruit), herbal (grass, dried herbs, thyme, basil, tarragon, rosemary, sage), floral, smoky, and spicy. Descriptors were previously selected by the panel while describing the Zelen and Pinela wine aromas. As this study mainly aimed to characterise the Zelen aromatic signature known for its herbal and spicy notes, the panel was previously trained on the corresponding descriptors using appropriate reference standards (Table S2).

4. Statistical analysis

4.1. Chemical analysis

The significance between samples analysed in surveys 1 and 2 was examined using one-way analysis of variance (ANOVA), and the means were separated using Fisher's least significant difference (LSD) test (different letters account for significant differences at $p \leq 0.05$) This approach was widely used in previously published studies on wine aromatic typicality (Schüttler *et al*., 2015; Antalick *et al*., 2015; Slaghenaufi *et al*., 2021). Shapiro–Wilk test was used to verify the normal distribution of the data. ANOVA was performed using Excel software (Microsoft Corporation, Redmond, WA, USA). Shapiro–Wilk test was performed by using XLSTAT 2023 (Addinsoft SARL, Paris, France).

4.2 Sensory analysis

Sensory data from the sorting task were first processed by performing a hierarchical cluster analysis (HCA). Then, dissimilarities between samples were analysed using non-

TABLE 4. Concentrations of volatile thiols measured in survey 1 in ng/L for 4-methyl-4-sulfanylpentan-2-one (4-MSP), 3-sulfanylhexanol (3-SH), and 3-sulfanylhexyl acetate (3-SHA). Means followed by different letters in a row are significant at p ≤ 0.05 (Fischer's LSD).

	Zelen (n = 10)	Pinela (n = 5)	Malvazija (n = 6)	Chardonnay (n = 3)	Sauvignon (n = 3)
4-MSP	1.0 ± 2.0[c]	4.0 ± 4.0[bc]	11.9 ± 5.0[b]	2.0 ± 3.0[c]	26.0 ± 6.0[a]
3-SH	414 ± 155[c]	665 ± 257[ab]	512 ± 323[bc]	972 ± 388[ab]	1154 ± 440[a]
3-SHA	1.9 ± 4.0[a]	9.5 ± 11[a]	7.7 ± 8[a]	15.9 ± 17[a]	<LOQ ± ND

metric multidimensional scaling (MDS) as described elsewhere (Suklje *et al*., 2015). The analysis of wine aromatic compounds aimed to identify potential relationships between wine composition and the effect of the variety on aroma. The correlations between wines, attributes, and chemical compounds were plotted on an MDS map. All the statistical analyses were performed by using XLSTAT 2023 (Addinsoft SARL, Paris, France) before the statistical analysis, the data were autoscaled.

RESULTS AND DISCUSSION

1. Wine selection

Published works on wine aromatic typicality have shown the importance of the selection of wines subject to sensory and chemical analyses (Ballester *et al*., 2005; Schüttler *et al*., 2015). In this context, the quality and relevance of selected samples are often more important than the number of samples. For instance, highly cited studies on Chardonnay, Sauvignon, and Riesling wine typicality were performed on 48, 18, and 30 commercial wines, respectively (Ballester *et al*., 2005; Parr *et al*., 2007; Schüttler *et al*., 2015). In each of these studies, wines were selected from two vintages to avoid the ageing effect between samples that could disturb the characterisation of varietal typicalities. Therefore, the same strategy was applied in the present work on three and four vintages for the survey 1 and 2, respectively. Another factor that influenced wine selection was the low number of commercially available Zelen wines on the market as the variety is only grown over 85 ha exclusively in Vipava Valley. Survey 1 analysed three varietal thiols in 27 wines originating from the Vipava Valley and other Slovenian wine regions including regional (Zelen, Pinela, and Malvasia Istriana) and international varieties (Chardonnay and Sauvignon blanc). The second survey was performed for 67 wine volatiles on 67 wines of the same varieties as in survey 1 but exclusively originating from Vipava Valley (Table 5). A wider selection was made for Zelen and Pinela wines to highlight the specific chemical composition of Zelen wines.

2. Are varietal thiols, esters, and C6-alcohols important for Zelen wine aroma?

The first survey analysed three varietal thiols in 27 wines originating from the Vipava Valley and other Slovenian wine regions including regional (Zelen, Pinela, and Malvasia Istriana) and international varieties (Chardonnay and Sauvignon blanc). Previous anecdotal evidence suggests that the concentrations of 3-SH and 3-SHA in Zelen wines could be as high as those in Sauvignon blanc. However, typical Zelen wine aromas are rarely affected by fruity notes reminiscent of varietal thiols, as in Sauvignon blanc wines. Zelen wines selected for this survey were described as typical by three local sommeliers. Table 4 shows that Zelen wines displayed the lowest average concentrations of 3-SH and 4-MSP in comparison to the other studied varieties. As expected, Sauvignon blanc wines were the richest in 4-MSP and 3-SH. Overall, the 3-SHA concentrations were very low (< 40 ng/L) in all samples. The wines analysed in this survey were 2 to 3 years old, and fast hydrolysis of 3-SHA has been reported in white wines (Makhotkina and Kilmartin, 2012). While it is possible to have high levels of varietal thiols in Zelen wines, it seems not to be a common trait of this variety but rather the result of winemaking techniques.

In a second survey, 67 wine volatiles, including esters and C6-alcohols among other odorants, were analysed in 67 wines originating from Vipava Valley (Table 5). Esters play a key role in the expression of wine fruity aromas, particularly in young white wines (Ribéreau-Gayon *et al*., 2006). They are mainly synthesised during alcoholic fermentation, but grape composition can also alter the wine ester composition (Antalick *et al*., 2015b). Zelen wines showed the lowest average concentration in some major esters such as the most important higher alcohol acetates and ethyl hexanoate, with significant differences in comparison to Pinela, another important regional variety planted in Vipava Valley (Table 5). The average concentration for ethyl esters of branched acids did not significantly differ between varieties. Varietal differences in wine ester composition have been reported in the literature (Hernández-Orte *et al*., 2002; Marais, 2003). Although the ester concentration is shaped during alcoholic fermentation and ageing, it seems that the varietal composition of must from Zelen grapes tends to limit the production of important esters during alcoholic fermentation.

C6-alcohols, such as hexanol and hexenol isomers, are reminiscent of freshly cut grass aroma and can contribute to the herbal character of some wines (Ribéreau-Gayon *et al*., 2006). Their relative composition depends on the variety (Versini *et al*., 1994). In our study, all the C6-alcohols were present at levels below the perception thresholds. Sauvignon blanc and Pinela wines showed the highest average concentrations among all the studied varieties, but the differences were not always significant. In contrast, Zelen wines significantly displayed the lowest concentrations of *Z*-3-hexenol, the most potent C6-alcohol. This result suggests that C6-alcohols are probably not specific chemical markers of Zelen wines.

TABLE 5. Concentrations of wine volatiles from survey 2 were measured in µg/L. One-way ANOVA was used to compare the data. Means followed by different letters in a row are significant at $p \leq 0.05$ (Fischer's LSD). (part. 1/2)

	Zelen (n = 25)	Pinela (n = 22)	Malvazija (n = 8)	Chardonnay (n = 4)	Sauvignon (n = 5)
Esters					
Higher alcohol acetates					
propyl acetate	20 ± 7^{a}	26 ± 14^{a}	27 ± 17^{a}	32 ± 14^{a}	24 ± 8^{a}
2-methylpropyl acetate	32 ± 13^{a}	39 ± 20^{a}	27 ± 16^{a}	36 ± 23^{a}	23 ± 6^{a}
phenylethyl acetate	116 ± 116^{a}	178 ± 110^{a}	104 ± 124^{a}	240 ± 347^{a}	178 ± 143^{a}
butyl acetate	1.1 ± 1.1^{a}	1.9 ± 1.8^{a}	2.2 ± 1.1^{a}	1.6 ± 0.8^{a}	1.9 ± 0.4^{a}
isoamyl acetate	846 ± 678^{b}	1604 ± 1334^{a}	1137 ± 1338^{ab}	1543 ± 2173^{ab}	1285 ± 1027^{ab}
hexyl acetate	12.0 ± 17.7^{b}	49.5 ± 61.2^{a}	19.0 ± 35.9^{ab}	38.7 ± 73.9^{ab}	55.0 ± 61.6^{ab}
z-3-hexenyl acetate	0.6 ± 0.3^{c}	2.2 ± 2.1^{b}	1.3 ± 1.9^{c}	1.7 ± 2.4^{c}	3.1 ± 3.3^{a}
E-2-hexenyl acetate	0.50 ± 0.03^{a}	1.6 ± 2.6^{a}	1.7 ± 2.7^{a}	0.53 ± 0.03^{a}	3.5 ± 5.0^{a}
octyl acetate	0.22 ± 0.02^{a}	0.4 ± 0.6^{a}	0.22 ± 0.04^{a}	0.2 ± 0.1^{a}	0.23 ± 0.04^{a}
Ethyl esters of fatty acids					
ethyl propanoate	165 ± 49^{a}	161 ± 52^{a}	204 ± 49^{a}	198 ± 31^{a}	182 ± 50^{a}
ethyl butanoate	226 ± 120^{a}	276 ± 115^{a}	290 ± 86^{a}	278 ± 99^{a}	280 ± 72^{a}
ethyl hexanoate	963 ± 485^{b}	1286 ± 588^{a}	1243 ± 512^{ab}	1349 ± 324^{ab}	1376 ± 270^{ab}
ethyl octanoate	1306 ± 594^{a}	1512 ± 612^{a}	1448 ± 554^{a}	1613 ± 541^{a}	1452 ± 294^{a}
ethyl decanoate	301 ± 221^{a}	345 ± 187^{a}	312 ± 202^{a}	357 ± 216^{a}	315 ± 122^{a}
ethyl pentanoate	2.1 ± 0.5^{a}	1.9 ± 0.5^{a}	2.0 ± 0.3^{a}	2.1 ± 0.4^{a}	2.1 ± 0.3^{a}
Ethyl esters of branched acids					
ethyl 2-methylpropanoate	101 ± 41^{a}	118 ± 77^{a}	138 ± 67^{a}	120 ± 35^{a}	112 ± 42^{a}
ethyl 2-methylbutanoate	20 ± 8^{a}	19 ± 11^{a}	33 ± 25^{a}	23 ± 13^{a}	19 ± 7^{a}
ethyl 3-methylbutanoate	29 ± 14^{a}	29 ± 20^{a}	51 ± 34^{a}	34 ± 18^{a}	30 ± 12^{a}
Ethyl 2-hydroxy-4-methylpentanoate	62 ± 32^{a}	57 ± 51^{a}	72 ± 42^{a}	57 ± 45^{a}	46 ± 14^{a}
ethyl phenylacetate	7.2 ± 2.5^{a}	9.6 ± 10.1^{a}	8.3 ± 3.9^{a}	11.1 ± 5.4^{a}	8.1 ± 5.0^{a}
Miscellaneous esters					
ethyl cinnamate	0.7 ± 0.2^{a}	0.6 ± 0.2^{a}	0.7 ± 0.3^{a}	2.6 ± 1.2^{a}	0.7 ± 0.2^{b}
ethyl dihydrocinnamate	0.2 ± 0.1^{b}	0.3 ± 0.2^{b}	0.2 ± 0.2^{b}	0.4 ± 0.3^{a}	0.3 ± 0.1^{b}
C6-alcohols					
hexanol	1075 ± 550^{a}	1277 ± 609^{a}	885 ± 282^{a}	858 ± 326^{a}	1197 ± 492^{a}
cis-3-hexenol	11 ± 9^{c}	45 ± 17^{b}	44 ± 18^{b}	36 ± 20^{b}	61 ± 47^{a}
trans-2-hexenol	14 ± 11^{a}	14 ± 17^{a}	22 ± 20^{a}	12 ± 5^{a}	8 ± 3^{a}
trans-3-hexenol	19 ± 10^{b}	56 ± 48^{a}	31 ± 17^{ab}	31 ± 13^{ab}	59 ± 34^{ab}
Volatile phenols					
4-ethyl phenol	56 ± 121^{a}	60 ± 158^{a}	40 ± 77^{a}	16 ± 23^{a}	9 ± 10^{a}
4-ethyl guaiacol	495 ±1017^{a}	192 ± 360^{a}	242 ± 474^{a}	25 ± 17^{a}	13 ± 16^{a}
methyl salicylate	14.3 ± 8.1^{a}	2.5 ± 2.3^{b}	4.0 ± 2.4^{b}	3.4 ± 1.2^{b}	3.1 ± 1.3^{b}
4-vinyl guaiacol	353 ± 127^{a}	284 ± 120^{b}	290 ± 129^{b}	177 ± 24^{c}	184 ± 32^{c}
4-vinyl phenol	90 ± 32^{a}	149 ± 54^{b}	120 ± 39^{a}	122 ± 48^{a}	154 ± 76^{b}
guaiacol	2.1 ± 0.4^{b}	2.1 ± 0.6^{b}	2.7 ± 0.8^{b}	3.3 ± 1.3^{a}	2.5 ± 0.7^{b}
Norisprenoids					
trans-β-damascenone	1.6 ± 1.2^{a}	1.1 ± 0.7^{a}	1.5 ± 0.9^{a}	0.7 ± 0.2^{a}	0.8 ± 0.8^{a}
α-ionone	0.04 ± 0.03^{a}	0.03 ± 0.03^{a}	0.03 ± 0.03^{a}	0.01 ± 0.01^{a}	0.025 ± 0.005^{a}
β-ionone	0.02 ± 0.01^{a}	0.03 ± 0.04^{a}	0.01 ± 0.01^{a}	0.011 ± 0.002^{a}	0.01 ± 0.01^{a}
TDN	0.60 ± 0.11^{a}	0.60 ± 0.07^{a}	0.69 ± 0.19^{a}	0.53 ± 0.11^{a}	0.68 ± 0.18a
vitispirane 1	1.4 ± 1.1^{a}	0.7 ± 0.6^{b}	1.6 ± 1.2^{a}	0.5 ± 0.5^{b}	2.1 ± 1.8^{a}
vitispirane 2	1.1 ± 0.6^{b}	0.9 ± 0.5^{b}	1.4 ± 0.8^{a}	0.8 ± 0.5^{b}	2.0 ± 1.3^{a}

TABLE 5. Concentrations of wine volatiles from survey 2 were measured in µg/L. One-way ANOVA was used to compare the data. Means followed by different letters in a row are significant at p ≤ 0.05 (Fischer's LSD). (part. 2/2)

Monoterpenes					
3-carene	0.068 ± 0.001[a]	0.068 ± 0.001[a]	0.068 ± 0.001[a]	0.067 ± 0.001[a]	0.067 ± 0.001[a]
α-terpinene	0.61 ± 0.12[a]	0.39 ± 0.02[c]	0.51 ± 0.09[b]	0.43 ± 0.05[bc]	0.44 ± 0.05[bc]
limonene	0.68 ± 0.28[a]	0.10 ± 0.04[c]	0.47 ± 0.28[b]	0.20 ± 0.11[bc]	0.16 ± 0.08[c]
1,4-cineol	0.22 ± 0.09[a]	0.13 ± 0.05[b]	0.15 ± 0.07[b]	0.13 ± 0.03[b]	0.25 ± 0.09[a]
eucalyptol	0.30 ± 0.10[a]	0.08 ± 0.08[b]	0.19 ± 0.10[b]	0.09 ± 0.03[b]	0.10 ± 0.05[b]
γ-terpinene	0.24 ± 0.08[a]	0.08 ± 0.01[c]	0.17 ± 0.06[b]	0.11 ± 0.03[bc]	0.12 ± 0.05[bc]
p-cymene	0.31 ± 0.10[a]	0.12 ± 0.02[c]	0.24 ± 0.08[a]	0.15 ± 0.05[b]	0.19 ± 0.07[ab]
α-terpinolene	0.46 ± 0.16[a]	0.14 ± 0.02[c]	0.34 ± 0.16[bc]	0.21 ± 0.09[b]	0.18 ± 0.04[b]
cis-rose oxide	0.12 ± 0.03[a]	0.11 ± 0.03[a]	0.12 ± 0.02[a]	0.12 ± 0.04[a]	0.12 ± 0.03[a]
trans-rose oxide	0.048 ± 0.003[a]	0.045 ± 0.005[a]	0.048 ± 0.003[a]	0.047 ± 0.004[a]	0.048 ± 0.004[a]
trans-linalool oxide	20 ± 29[a]	8 ± 12[a]	27 ± 51[a]	7 ± 4[a]	19 ± 25[a]
cis-linalool oxide	14 ± 25[a]	4 ± 8[a]	14 ± 28[a]	3 ± 2[a]	9 ±12[a]
linalool	52 ± 35[a]	6 ± 4[c]	34 ± 30[b]	12 ± 10[c]	6 ± 7[c]
4-terpineol	1.5 ± 0.8[b]	0.4 ± 0.2[c]	1.3 ± 1.2[b]	0.4 ± 0.2[c]	2.4 ± 3.1[a]
hotrienol	1.5 ± 1.3[a]	0.6 ± 0.5[a]	2.2 ± 1.4[a]	1.7 ± 2.1[a]	0.9 ± 0.6[a]
α-terpineol	61 ± 25[a]	7 ± 4[c]	38 ± 24[b]	10 ± 7[c]	14 ± 10[c]
citronellol	7.9 ± 6.2[a]	5.2 ± 4.7[a]	5.8 ± 4.7[a]	4.3 ± 1.8[a]	4.1 ± 3.1[a]
nerol	6.8 ± 3.9[a]	2.9 ± 0.6[b]	4.6 ± 2.4[b]	3.3[b] ± 0.9[a]	2.6 ± 0.5[b]
Sesquiterpene					
trans-nerolidol	0.3 ± 0.2[a]	0.17 ± 0.05[a]	0.20 ± 0.08[a]	0.15 ± 0.03[a]	0.2 ± 0.1[a]
cis-nerolidol	0.7 ± 0.3[a]	1.0 ± 0.8[a]	0.6 ± 0.2[a]	0.7 ± 0.3[a]	0.4 ± 0.1[a]
Additional monoterpenes					
β-myrcene*	0.27 ± 0.17[a]	0.04 ± 0.02[c]	0.18 ± 0.15[b]	0.09 ± 0.06[c]	0.04 ± 0.03[c]
β-phellandrene*	0.010 ± 0.005[a]	0.001 ± 0.001[c]	0.005 ± 0.004[b]	0.003 ± 0.002[c]	0.002 ± 0.002[c]
cis-ocimene*	0.120 ± 0.078[a]	0.016 ± 0.009[c]	0.076 ± 0.064[b]	0.038 ± 0.027[c]	0.015 ± 0.012[c]
trans-ocimene*	0.0014 ± 0.001[a]	0.0003 ± 0.0002[c]	0.0009 ± 0.001[b]	0.0003 ± 0.0001[c]	0.001 ± 0.0003[c]
2,4,6-octatriene-2,6-dimethyl_1*	0.010 ± 0.006[a]	0.002 ± 0.001[c]	0.006 ± 0.005[b]	0.003 ± 0.002[c]	0.001 ± 0.001[c]
2,4,6-octatriene-2,6-dimethyl_2*	0.012 ± 0.007[a]	0.002 ± 0.001[c]	0.008 ± 0.006[b]	0.004 ± 0.003[c]	0.002 ± 0.001[c]
habanene*	0.032 ± 0.010[a]	0.013 ± 0.002[b]	0.027 ± 0.011[a]	0.016 ± 0.005[b]	0.018 ± 0.005[b]
cosmenene*	0.010 ± 0.006[a]	0.003 ± 0.002[b]	0.009 ± 0.006[a]	0.003 ± 0.004[b]	0.004 ± 0.001[b]

*Semi-quantitative data (relative peak area).

3. The terpenes diversity in Zelen wine composition

Terpenes and norisoprenoids were analysed in the second survey, which included 29 monoterpenes, two sesquiterpenes, and six norisoprenoids. Norisoprenoid wine composition was only slightly influenced by the variety. Zelen wines tended to display higher levels of β-damascenone than Chardonnay and Sauvignon blanc but these differences were not statistically significant. In contrast, concentrations of vitispirane 1 and 2 were significantly higher in Zelen than in Pinela and Chardonnay wines (Table 5). The other norisoprenoids did not show any trend when their contents were compared between varieties. The norisoprenoids measured in this study did not seem to be directly involved in the aromatic uniqueness of Zelen wines.

In contrast, important varietal effects were observed within the group of terpenes (Table 5). Zelen wines were the richest in monoterpenes among all the varieties. The first group of compounds that was particularly found in higher concentrations in Zelen wines were monoterpenes with simple hydrocarbon structures, namely terpinene isomers, limonene, p-cymene, and terpinolene. Hence, the concentration levels were 2 to 7-fold higher in Zelen wines in comparison to Pinela, Chardonnay, and Sauvignon blanc wines. The profile of hydrocarbon terpenes of Malvasia Istriana wines was more similar to that of Zelen but with lower concentration levels. The sensory impact of these compounds on wine aroma has not been well-documented in the literature. The perception threshold for limonene in water was reported to be in the range of 200–500 µg/L according to the enantiomer (Boelens *et al*., 1993). The highest concentration observed in the present study was measured at 1.1 µg/L in Zelen wines.

However, a recent study has suggested that concentration of limonene as low as 2 µg/L could influence white wine aroma when limonene was associated with other terpenes (Chigo-Hernandez & Tomasino, 2023). Therefore, as a group of seven volatiles, including 1,4-cineole and 1,8-cineole, were also measured at higher concentrations in Zelen wines, the contribution of hydrocarbon monoterpenes to Zelen aromatic typicality through perceptive interactions cannot be excluded. The importance of monoterpenes for the Zelen aromatic profile was confirmed with the group of terpenols (Table 5). Zelen wines were particularly rich in linalool and α-terpineol compared to Pinela, Chardonnay, and Sauvignon blanc wines. Once again, Malvasia Istriana wines had a terpenol profile similar to that of Zelen in a slightly lower concentration range. The average concentrations were calculated at 52 µg/L and 61 µg/L in Zelen wines for linalool and α-terpineol, respectively, and the maximal concentrations were measured up to 100 µg/L for both compounds. These values are in agreement with previously published data (Bavčar *et al*., 2016). With a sensory threshold of approximately 15 µg/L in model wine, linalool can probably contribute directly to the Zelen aromatic profile. Linalool is reminiscent of coriander seed, lavender, and rose, and plays a major role in the aroma of Muscat and Gewürztraminer wines (Ribéreau-Gayon *et al*., 2006). Linalool is also an important contributor of aromatic herbs (De Martino *et al*., 2021). The threshold value of α-terpineol in wine is approximately 400 µg/L and does not directly contribute to the Zelen wine aroma (Ribéreau-Gayon *et al*., 2006). Nevertheless, recent studies have demonstrated that the addition of 30 µg/L of α-terpineol to a complex mixture of terpenes could enhance the ginger and lime aroma of Pinot Gris wine (Chigo-Hernandez & Tomasino, 2023). Therefore, the indirect influence of α-terpineol on Zelen wine aroma cannot be excluded even though the wine matrix is more complex than the model solutions used in the previously cited study. Future work is warranted to clarify this hypothesis. Nerol was also present at higher levels in Zelen wines, but its contribution to Zelen aroma is unlikely, because the highest concentration measured in Zelen was 25-fold lower than its olfactory threshold measured in wine (Ribéreau-Gayon *et al*., 2006).

4. A rare chemical profile in volatile phenols

A total of six volatile phenols were analysed in the second survey. In most of the wines 4-ethylphenol (4-EP) and 4-ethylguaiacol (4-EG) were present at concentrations well below their sensory thresholds reported at 600 µg/L and 110 µg/L, respectively (Chatonnet *et al*., 1992) (Table 5). However, a dozen of wines displayed high concentrations of these volatile phenols, particularly in the case of 4-EG for which seven Zelen, five Pinela and one Malvasia Istriana wines had concentrations above its perception threshold. The highest concentrations in 4-EG were measured in Zelen wines with values up to 3200 µg/L. Ethylphenols (EPs) impart to wines unpleasant sensory attributes such as phenolic, animal, and stable aromatic notes (Chatonnet *et al*., 1992). In wine, their presence is mainly due, although not exclusively, to the conversion of hydroxycinnamic acid precursors, p-coumaric and ferulic acids, respectively, by yeast Dekkera/*Brettanomyces bruxellensis* to the corresponding vinyl phenols (VPs) by the hydroxycinnamate decarboxylase. Through reduction by vinylphenol reductase, VPs are converted to the corresponding EPs. In conventional white wines, EPs are rarely present at high levels as the risk of spoilage by *Brettanomyces* yeast is limited by higher acidity and shorter ageing in wooden barrels (Milheiro *et al*., 2019). However, when white wines display high pH due to prolonged skin-contact maceration and are aged in barrels, EPs levels increase (Lukić *et al*., 2015). Numerous winemakers from Vipava Valley perform a short skin-contact maceration in Zelen wine production to enhance its varietal character. Some winemakers combine this approach with spontaneous fermentation and very low sulfite addition. Interestingly, the wines that displayed the highest concentration in 4-EP and 4-EG were made using this protocol of vinification which is riskier regarding the contamination by *Brettanomyces*. However, in their review of EPs in red wines, Milheiro *et al*. (2019) reported that 4-EP was always present in higher concentrations than 4-EG in the case of spoilage with Brettanomyces. Cabrita *et al*. (2012)

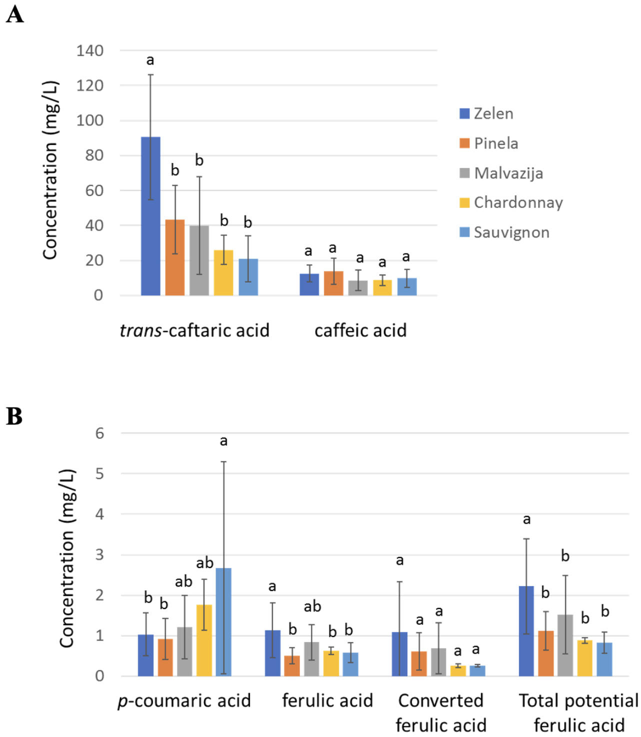

FIGURE 1. Concentrations of major (A) and minor (B) hydroxycinnamic acids measured in wines from survey 2. Converted ferulic acid is the equivalent ferulic acid concentration calculated from 4-ethylguaiacol and 4-vinylguaiacol concentrations in wines. Total potential ferulic acid is the sum of ferulic acid and converted ferulic acid concentrations. One-way ANOVA was used to compare data. Different letters in a column represent significantly ($p \leq 0.05$) different concentrations expressed in milligrams per litre. Standard errors were used for the error bars.

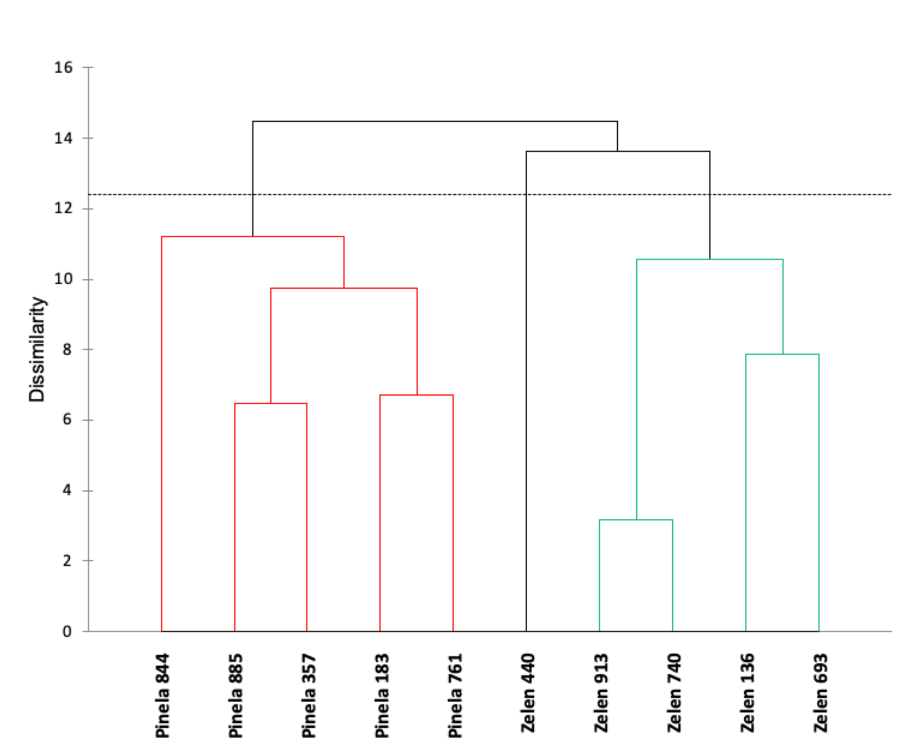

FIGURE 2. Hierarchical cluster analysis (HCA) of sorting task data.

calculated a similar conversion rate of p-coumaric acid into 4-EP as ferulic acid converts into 4-EG. On the other hand, in the present study, the wines contaminated by EPs were much richer in 4-EG than in 4-EP. While *Brettanomyces* is the main source of EPs in wine, other microorganisms can produce perceptible amounts of these volatiles. For instance, Saez *et al*. (2011) found that the yeast species *Pichia manshurica* isolated from Argentinian red and white wines was able to produce a much higher amount of 4-EG than 4-EP in red wine even when microorganisms were present in a non-cultivable state. Further research investigating the origin of EPs in skin-contact white wines is warranted.

Unlike EPs, many yeast species, including *Saccharomyces cerevisiae*, can produce 4-vinylphenol (4-VP) and 4-vinylguaiacol (4-VG) in white wines (Grando *et al*., 1993). Zelen wines displayed the lowest average concentration in 4-VP but the highest in 4-VG (Table 5). The highest concentration of 4-VP was measured at 278 µg/L in Sauvignon blanc, a value which is below the sensory threshold of this compound in wine (710 µg/L) (Chatonnet *et al*., 1992). In contrast, 92 % of Zelen wines had 4-VG contents higher than 200 ug/L, which corresponds to the reported threshold in wine (Grando *et al*., 1993). This proportion was lower for the other varieties with 75 %, 62 %, 25 % and 0 % for Malvasia, Pinela, Chardonnay and Sauvignon blanc wines respectively. The highest concentrations of 4-VG were measured in Zelen wines with a peak at 594 µg/L. While some authors consider that 4-VG contributes to some white wine off-flavours described as "band-aid" and a "gouache-like odour" (van Wik & Rogers, 2000), other authors suggested that 4-VG contributed to the pleasant spicy flavour of Gewürztraminer wines (Versini, 1985). Zelen wines often display some spicy, smoky-phenolic aromas, and this result suggests that 4-VG could potentially play a role in this specific aromatic profile.

To investigate the trend of Zelen wines displaying higher levels of 4-VG, four hydroxycinnamic acids were analysed using HPLC-UV. Figure 1 shows that Zelen wines are the richest in ferulic acid, a known 4-VG precursor in wine (Chatonnet *et al*., 1992). The difference from the other varieties is even more important when the proportion of ferulic acid already converted into volatile phenols is considered (Figure 1). In that case, Zelen wines showed an average concentration of total potential ferulic acid twice that of *p*-coumaric acid. Such a profile of hydroxycinnamic acids seems to be highly specific to the Zelen variety, especially in comparison to international varieties that generally contain higher levels of *p*-coumaric acid than ferulic acid, as observed in the present study (Figure 1) (Pour Nikfardjam *et al*., 2009). Therefore, the trend of Zelen wines to display a higher concentration of 4-VG seems to be a varietal characteristic. It is worth noting that Zelen wines were also very rich in *trans*-caftaric acid. This major hydroxycinnamic acid is not metabolised into volatile phenols but can be easily converted into quinone in grape must which in turn can oxidise varietal thiols (Ribéreau-Gayon *et al*., 2006). This could potentially contribute to the general lower concentrations of grape-derived thiols measured in commercial Zelen wines (Table 4).

Finally, methyl salicylate (MeSA) was one of the most important chemical markers to distinguish Zelen from the other varieties. The average concentration of MeSA was from 3.5 to 6-fold higher in Zelen wines in comparison to the other wines and ranged from 4.5 to 39 µg/L with an average of 14.3 µg/L. MeSA has a characteristic aroma of wintergreen oil, spicy and minty, and its odour threshold in white wine was recently reported at 38 µg/L (Slaghenaufi *et al*., 2022). Nevertheless, 20 % of the panellists in that study were able to detect the presence of MeSA in white wine from 14 µg/L. This result suggests that MeSA could potentially be a marker of Zelen

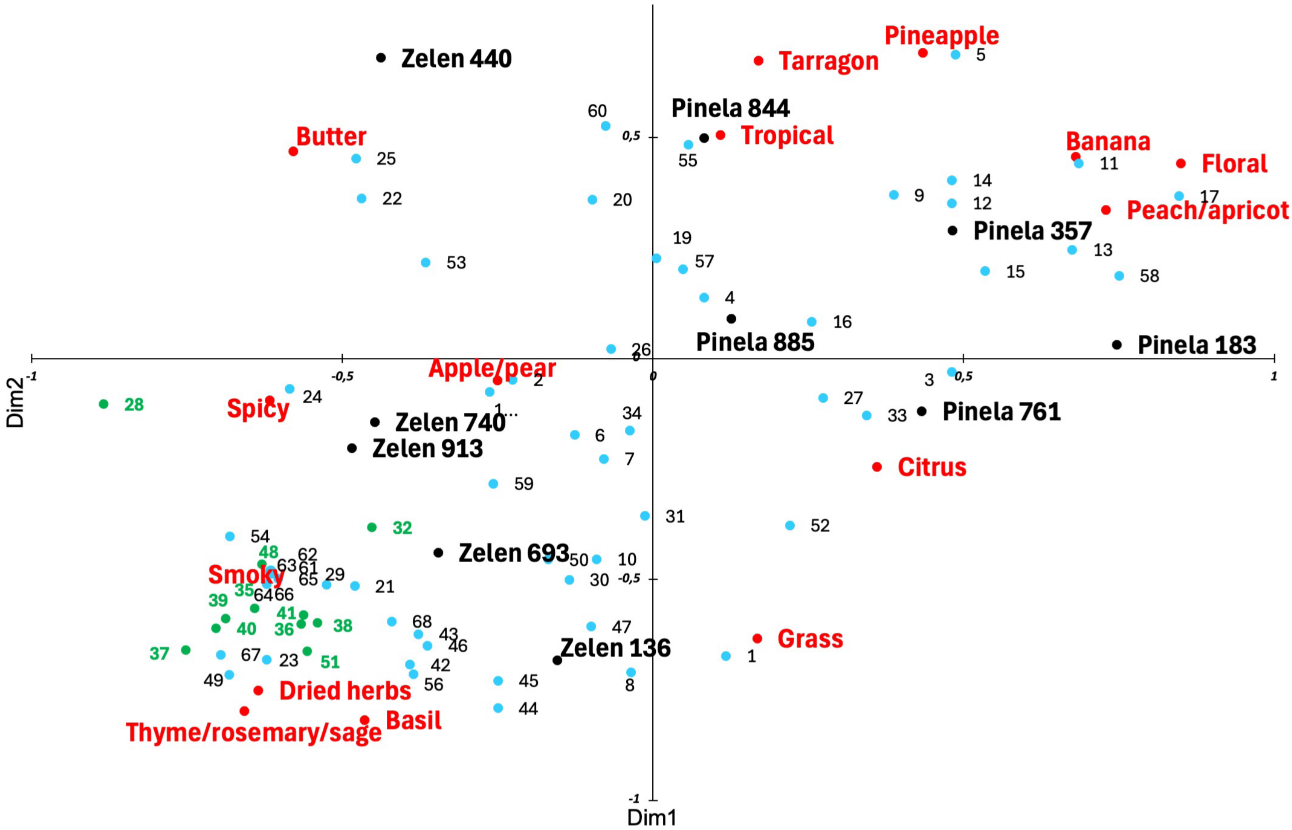

FIGURE 3. Two-dimensional multi-dimensional scaling (MDS) configuration of the Pinela and Zelen wines (black) and correlations of the sensory terms (red) and chemical compounds (blue and green) with the dimensions. The numbers in green correspond to monoterpenes and volatile phenols associated with Zelen wines in survey 2. The numbers correspond to the following compounds: 1. ethyl propanoate; 2. ethyl isobutyrate; 3. propyl acetate; 4. isobutyl acetate; 5. ethyl butyrate; 6. ethyl 2-methylbutyrate; 7. ethyl isovalerate; 8. butyl acetate; 9. isoamyl acetate; 10. ethyl valerate; 11. ethyl hexanoate; 12. hexyl acetate; 13. Z-3-hexenyl acetate; 14. E-2-hexenyl acetate; 15. hexanol; 16. E-3-hexenol; 17. Z-3-hexenol; 18. E-2-hexenol; 19. ethyl octanoate; 20. octyl acetate; 21. ethyl leucate; 22. ethyl decanoate; 23. ethyl phenylacetate; 24. phenylethyl acetate; 25. ethyl dodecanoate; 26. ethyl dihydrocinnamate; 27. ethyl cinammate; 28. methyl salicylate; 29. guaiacol; 30. 4-ethylguaiacol; 31. 4-ethylphenol; 32. 4-vinylguaiacol; 33. 4-vinylphenol; 34. 3-Carene; 35. α-terpinene; 36. limonene; 37.1,4-cineol; 38. eucalyptol; 39. γ-terpinene; 40. p-cymene; 41. α-terpinolene; 42. cis-rose oxide; 43. trans-rose oxide; 44. *trans*-linalool oxide; 45. *cis*-linalool oxide ; 46. vitispirane 1; 47. vitispirane 2; 48. linalool; 49. 4-terpineol; 50. hotrienol; 51. α-terpineol; 52. TDN; 53. citronellol; 54. nerol; 55. *trans*-β-damascenone; 56. *trans*-geraniol; 57. α-ionone; 58. β-ionone; 59. *trans*-nerolidol; 60. *cis*-nerolidol; 61. β-myrcene; 62. β-phellandrene; 63. β-E-ocimene; 64. β-Z-ocimene; 65. 2,4,6-octatriene-2,6-dimethyl 1; 66. 2,4,6-octatriene-2,6-dimethyl 2; 67. habanene; 68. cosmene.

aroma typicality. MeSA is a grape-derived compound present mainly in the form of glycosides in grapes (Carlin *et al*., 2019; Slaghenaufi *et al*., 2022). This compound has recently been detected at suprathreshold levels in red wines produced under specific conditions (Poitou *et al*., 2021; Pelonnier-Magimel *et al*., 2022). In contrast, in white wines, MeSA concentration only reached or exceeded the levels found in Zelen in the Italian Verdicchio and Lugana wines made from Turbiana. In those wines, MeSA concentrations ranged from 6.7 to 200 µg/L, while it was below 5 µg/L in all other wines (Slaghenaufi *et al*., 2022). In the present study, 92 % of Zelen wines displayed MeSA concentrations above 5 µg/L, whereas only five wines that were not made from Zelen but from Pinela and Malvasia Istriana had MeSA concentrations above 5 µg/L. Pelonnier-Magimel *et al*. (2022) recently demonstrated that MeSA is a marker of Bordeaux red wines made without sulphites. Interestingly, Pinela and Malvasia Istriana wines with MeSA contents above 5 µg/L were all made with a low addition of sulphite just before bottling. The production of MeSA from microorganisms during the ageing of sulphite-free wine cannot be excluded and requires further investigation, as suggested by Pelonnier-Magimel *et al*. (2022). However, the MeSA concentration of these five wines did not exceed 10.3 µg/L, unlike 70 % of Zelen wines which displayed higher concentration suggesting that differences in MeSA contents between wines mainly depended on the variety. Although Zelen and Turbiana are different varieties, a potential genetic link between them should also be subject to future research.

Overall, the chemical markers of Zelen commercial wine aromatic profiles could clearly be highlighted with a simple approach irrespective of the vintage and producers. This reflects the original aromatic fingerprint of the Zelen variety and the winemaking techniques favouring the Zelen varietal expression. The relevance of the wine selection also made the use of advanced statistical tools unnecessary to highlight the specific chemical composition of Zelen commercial wines. Previously published studies on wine aromatic typicality have used the same approach (Schüttler *et al*., 2015; Antalick *et al*., 2015a; Slaghenaufi *et al*., 2021).

5. Sensory characterisation of Zelen commercial wine aromas and identification of potential chemical markers

To confirm the potential relationship between the compounds of interest highlighted in the chemical surveys and Zelen aromatic profile, a sorting task analysis was performed. The panellists had to evaluate whether there were olfactory similarities between Zelen wines, which were different from Pinela counterparts. Wine volatiles were also analysed to determine if the chemical differences matched the second survey. The sensory data were subjected to hierarchical cluster analysis (HCA), and the results are shown in Figure 2. First, the quality of the panel used for the analysis was assessed. Two samples (913 and 740) of the same Zelen wine from different vintages were correctly grouped together, suggesting the effectiveness of the panel. Furthermore, it can be observed the existence of three distinct clusters, one formed exclusively by Pinela wines, one by Zelen wines except for Zelen 440 sample that formed a separate cluster.

To assess the aroma profile of wines and their potential link with volatile composition, the frequencies of citation of each descriptor for each sample were submitted to non-metric multi-dimensional scaling (MDS) using the volatile concentrations as supplementary quantitative variables (Figure 3). The Zelen and Pinela wine samples were well separated into two clusters on the first dimension while the Zelen 440 sample stands alone separate from the other two groups, confirming the results that emerged from the HCA. The first axis, accounting for 47.82 % of the total variance, clearly separates Zelen and Pinela wines, while the second axis separates sample 440 from all other Zelen samples, explaining 18.15 % of the total variance. Pinela wines were characterised mostly by fruity aroma notes going from banana, pineapple, and tropical fruit to citrus descriptors. In contrast, Zelen wines were associated with dried herbs, thyme, rosemary, sage, and more generally spicy odour notes. These observations highlight the existence of specific olfactory spaces related to the two varieties, with Zelen showing a more specific herbal profile and Pinela a more common fruity profile. The Zelen 440 sample was considered an outlier because it was clustered alone and appeared to be highly characterised by specific olfactory notes, particularly butter, not associated with the other wine samples.

Fruity-driven Pinela wines were mainly correlated with major esters known to be important contributors to the fruity aroma in white wines. This also confirms that Zelen wines generally show lower concentrations of these compounds than Pinela (Table 5). In contrast, Zelen wines were associated with terpenes, methyl salicylate, and 4-VG, confirming the results of the second survey (Figure 5). Terpenes correlated to Zelen wine aroma were the same as those listed above, including some hydrocarbon monoterpenes and terpenols (Figure 3). As can be seen in Figure 3, they were well correlated on MDS with descriptors such as dried herbs, thyme, rosemary, basil, and smoky. The descriptor "smoky" was also correlated with guaiacol and 4-VG to a lesser extent, imparting a smoky/phenolic aroma to white wines. Guaiacol concentrations were well below its perception threshold in wine and varietal differences were not important (Parker *et al*., 2012). On the other hand, 4-VG was systematically present at higher concentrations in Zelen wines than in Pinela wines, except for Zelen 440 and Pinela 761 originating from the same producer (Table S3). The highest 4-VG concentration measured was 1044 µg/L in Zelen 913, suggesting that this compound can potentially play a role in the smoky notes typical of Zelen wines. Methyl salicylate correlated well with the descriptor "spicy" and was measured between 10 and 28 µg/L in Zelen while concentrations in Pinela were always below 2 µg/L (Table S3). While these values are below the olfactory threshold, it has been shown that some individuals can detect the presence of methyl salicylate at such concentrations (Slaghenaufi *et al*., 2022). This result confirms that methyl salicylate is a good chemical marker of the Zelen variety and could potentially contribute to its typical spicy notes, probably through perceptive interactions with other compounds.

6. Isolation and characterisation of herbaceous aromatic fractions

To investigate the potential presence of other aromatic compounds that could contribute to Zelen wine aroma, a comparison of the different aromatic fractions obtained by HPLC fractionation between one typical Zelen wine and one Pinela wine was performed by olfactory evaluation. Sensory analysis carried out by two trained panellists

TABLE 6. Olfactive description of HPLC fractions and the corresponding wines.

Fractions	Zelen wine	Pinela wine
1-2	Water/Ethanol	Water/Ethanol
3-6	Butter	Butter
7	Burnt caramel	Burnt caramel
8-11	Marzipan	Marzipan
12	Floral	Floral
13	Herbal	Floral
14	Dried herbs	Winy, Fruity
15-16	Fruity, Leather	Fruity, Leather
17	Fruity	Fruity
18	Banana	Banana
19-20	Cooked apple, Tobacco	Cooked apple, Tobacco
21-25	Ethanol	Ethanol

showed that most of the aromatic fractions of Zelen and Pinela wines were similarly described excluding fractions 13 and 14, which were different (Table 6). In Pinela wine, these two fractions were perceived as floral and winy with some fruity undertone, whereas in Zelen wine, they were described as herbal and reminiscent of dried herbs typical of the Zelen variety. However, this cannot be considered a robust sensory characterisation of Zelen aromatic fractions due to the restricted number of panellists. On the other hand, the observation made by both trained panellists provided relevant information for the chemical investigation of aromatic fractions potentially specific to the Zelen variety. HS-SPME-GC/MS analysis of these two fractions revealed that they contained a complex mixture of hydrocarbon monoterpenes, including some compounds already identified as potential markers of Zelen wines, such as terpinene isomers, limonene, terpinolene, and cineole. Interestingly, eight new hydrocarbon monoterpenes were also identified in these fractions through their mass spectra with a good confidence level: β-myrcene, β-phellandrene, E-β-ocimene, Z-β-ocimene, two 2,4,6-octatriene-2,6-dimethyl isomers, and habanene and cosmene. GC retention times also matched the reported retention indices when compared to other quantified monoterpenes. Their odours are classified as herbaceous and reminiscent of dried herbs and different types of Mediterranean herbs and spices (www.thegoodscentscompany.com).

Therefore, regarding the potential impact of these new monoterpenes on Zelen wine aroma, chromatograms from the second survey of wine volatiles were processed again to assess their levels using semi-quantitative analysis. The values of their relative peak areas were added to Table 5 under the group “additional monoterpenes.” The wine content of these monoterpenes followed the same trend as that of other hydrocarbon monoterpenes, with the highest content found in Zelen wines. It was particularly obvious for β-myrcene, β-phellandrene, E-β-ocimene, Z-β-ocimene and both 2,4,6-octatriene-2,6-dimethyl isomers with concentrations 3 to 8-fold higher in Zelen than Pinela, Chardonnay and Sauvignon blanc wines.

Monoterpene concentrations in Malvasia Istriana were approximately 50 % lower than those measured in Zelen wines. Similar results were found when comparing Zelen and Pinela wines used for the sorting task, where they were all well correlated to the cluster of Zelen wines (compounds 61 to 68) (Figure 3). While this result appears promising to explain Zelen aromatic typicality, some quantitative analysis and appropriate sensory evaluation will have to investigate the sensory impact of these monoterpenes in a mixture on white wine aroma.

CONCLUSIONS

Zelen wine’s aromatic profile was characterised for the first time confirming empirical observations describing Zelen aromatic signature with typical notes of dried herbs, spices, and smoky undertones. The chemical markers of Zelen commercial wine’s aromatic profile could be clearly highlighted with a simple statistical approach irrespective of vintage and producers. This unique aromatic profile was connected with the presence of an original mixture of volatile phenols and different monoterpenes. Hence, a large group of hydrocarbon monoterpenes seems to be involved in the perception of the typical dried herbs aroma in Zelen wines. The sensory impact of most of those compounds in wine is still poorly documented. Considering the high levels of linalool and α-terpineol measured in Zelen wines, perceptive interactions within this rich group of terpenoids are not excluded. Future chemical and sensory analyses will have to address this topic. On the other hand, the presence of 4-vinylguaiacol and methyl salicylate potentially contribute to the spicy and smoky character of Zelen wine aroma. Methyl salicylate was particularly associated with Zelen wines compared to other white varieties grown in the Vipava Valley. The presence of volatile phenols as varietal markers of Zelen wine requires further investigation to better understand how winemaking protocols influence the volatile phenol composition of white wines.

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