Differentiation of Commercial PDO Wines Produced in Istria (Croatia) According to Variety and Harvest Year Based on HS-SPME-GC/MS Volatile Aroma Compound Profiling

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Summary

To differentiate monovarietal wines made from native and introduced varieties in Istria (Croatia), samples of Malvazija istarska, Chardonnay and Muscat yellow from two harvest years (2013 and 2014) were subjected to headspace solid-phase microextraction and gas chromatographic/mass spectrometric analysis (HS-SPME-GC/MS) of volatile aroma compounds. Significant effects of variety and harvest year were determined, but their interaction complicated the differentiation. Particular compounds were consistent as markers of variety in both years: nerol for Malvazija, ethyl cinnamate and a tentatively identified isomer of dimethylbenzaldehyde for Chardonnay, and terpenes for Muscat yellow. Wines from 2013 contained higher concentrations of the majority of important volatiles. A 100 % correct differentiation of Malvazija istarska and Chardonnay wines according to both variety and harvest year was achieved by stepwise linear discriminant analysis.

Key words: monovarietal wine, Croatia, volatile aroma compounds, variety, harvest year, HS-SPME-GC/MS

Introduction

Aroma is probably the most important organoleptic characteristic that defines the typicity and quality of wine. It is one of the crucial factors that determine its market value and price, and a key attribute for consumer preference. Wine aroma results from the occurrence of several hundreds of odouriferous volatile compounds originating from grapes (primary or varietal aromas), compounds produced in fermentation (secondary or fermentation aromas), and, in aged wines, compounds produced during maturation (tertiary aromas) (*1*). Volatile aroma compounds found in wine pertain to different chemical classes, such as monoterpenes, norisoprenoids, higher alcohols, fatty acids, esters, aldehydes, ketones, benzenoids, *etc.* (*2*), and occur in concentrations ranging from ng/L to a few hundreds of mg/L (*3*). The final aroma of a wine is a result of complex interactions between several factors, such as geographical location of the vineyard (*4*), which is linked

to soil and climatic conditions (*5*), harvest year (*6,7*), yeast strain (*8*), production parameters (*9*), *etc.* Particular attention has been devoted to the influence of varietal origin, and several studies have focused on the identification of volatile compounds typical for different varieties, important for the expression of varietal characteristics in wine (*10–20*). The knowledge of the chemical (volatile aroma) composition of varietal wines may give opportunities to producers to deeper understand the phenomena they observe in practice and control the production with greater efficiency to obtain wines with a more pronounced varietal typicity and higher quality. It may enable a proper characterisation and differentiation of varietal wines, attributing them an added marketing value. Wine is globally consumed, and it is a food commodity of relatively high commercial value and importance to the economy of many world countries. Therefore, wine authenticity control, among others, in terms of varietal origin characterisa-

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tion and differentiation, is continuously required to detect adulteration and to improve wine quality (21).

At this moment, the Republic of Croatia is the latest state that has joined the European Union. Like many other Mediterranean countries, it has a vitivinicultural tradition that is centuries long, with today a relatively developed wine industry and rather interesting domestic grape varieties. The quality of Croatian wines in recent years is rapidly and constantly improving, which resulted in significant success on the national and international markets and quality competitions. Their reputation has recently been strengthened by gaining European Protected Designations of Origin (PDO), which certify their authenticity and the connection of their quality with varietal and territorial origin. In the last few decades, Croatian domestic varieties, linked to specific terroirs and adapted to the local environmental conditions, have been used to produce original and high-quality wines and compete on the market with widely spread international varieties to attract consumers, nowadays more and more motivated by marketing attributes and new wine types rather than just pleasant aroma and taste (7).

From a scientific point of view, Croatian wines are poorly characterised when compared to other European and world wines. Because of the lack of objective scientific information, knowledge on the varietal typicity of wines from native Croatian grape varieties is still on an informal level. For example, there is published data on the composition of wines made from Malvazija istarska, the most spread and important native white grape variety in Croatia grown principally in the region of Istria (9,22–25), but the volatile compounds and their particular concentrations, indicators of its varietal origin and drivers of its varietal typicity, on the basis of which it might be distinguished and differentiated from other monovarietal white wines, are still unknown. On the other hand, Chardonnay is a globally spread and known variety, and its wine aroma has been investigated extensively (26–30). Despite many characterisation studies, Chardonnay has rarely been directly confronted with, compared to, and differentiated from other white wines of similar typology, especially from this part of Europe. The compounds responsible for typical Chardonnay aroma have been identified (26–30), but it is still not known if the amounts found are specific for this variety and can differentiate it from other monovarietal wines. A small number of previous differentiation attempts were limited in that they were based only on fermentation-derived compounds (31), comparison with mostly red wines (32), or used only *m/z* fragments after direct injection in an electrospray ionisation Fourier transform mass spectrometer (ESI-FT-MS), without the identification of volatile compounds as discriminating variables (33). Chardonnay wines in question were produced in South Africa, Brasil and Chile, respectively. Wines made from Muscat yellow grapes, another important variety in Croatia especially in the region of Istria, were globally studied extremely rarely, except those produced in Italy, which were investigated extensively, but relatively long ago (34–36).

The main objective of this study is to determine the differences in the composition of volatile aroma compounds between wines made from three important white grape varieties in the Istria region of Croatia (PDO Croatian Istria), through the combined use of HS-SPME-GC/MS profiling with univariate and multivariate statistics. The aim is to contribute to the knowledge on the varietal typicity of the domestic Malvazija istarska wine, as well as its differentiation from wines obtained from internationally known Chardonnay and Muscat yellow wines produced in the same area. Comparison between native Malvazija istarska and introduced Chardonnay is especially interesting and important because these are competing varieties used in Istria to produce typologically similar wines. Further, among the local professional wine community, Malvazija istarska wines are often linked to a so-called subtle 'muscat-like character'. The comparison with Muscat yellow wines in this study might help to clarify this attribute. Interaction of varietal origin with other factors of influence, namely harvest year, may complicate the characterisation, differentiation and authentication of monovarietal wines (37). For this reason, wines from two consecutive harvest years, significantly different with respect to climatic conditions, were included in this study.

Materials and Methods

Wine samples and harvest years

Samples of typical fresh, young, unoaked wine Malvazija istarska, Chardonnay and Muscat yellow, with Protected Designation of Origin (PDO Croatian Istria, traditional term: Quality wines), produced by standard winemaking technology (destemmed, crushed and mashed grapes, without or up to 24-hour skin contact, inoculation with commercial *Saccharomyces cerevisiae* yeast, and fermentation in stainless steel tanks at temperatures lower than 18 °C) were voluntarily consigned by the local producers in the Istria region of Croatia. After an informal preliminary sensory assessment of a larger number of wines, the most typical samples were chosen by a consensus of professional, highly experienced and certified wine tasters from the Institute of Agriculture and Tourism, Poreč, Croatia, on the basis of their personal varietal typicity concept. Five samples of each variety, from each of the two consecutive harvest seasons (2013 and 2014), were collected (total of 30 wines). The two studied harvests were rather different considering climatic conditions. According to the Croatian Meteorological and Hydrological State Institute, in the period from April to September 2013, the sum of the effective temperatures was 1806 °C, while the total rainfall amounted to 359 mm. Most of the rainfall was recorded in August (112 mm). In the same period in 2014, the sum of the effective temperatures was lower, 1683 °C, while the total rainfall was much higher: 546 mm. Most of the rainfall was recorded in July (154 mm) and September (128 mm). Because of the lower temperatures and lots of rain, 2014 was characterised by problematic and late ripening.

Chemical standards and standard solutions of volatile aroma compounds

Pure standards of individual volatile aroma compounds were purchased from Merck (Darmstadt, Germany), Sigma-Aldrich (St. Louis, MO, USA), and Fluka (Buchs,

Switzerland). Stock standard solutions were prepared in ethanol. Working standard solutions were prepared by dilution of stock standard solutions in synthetic wine containing 12 % of ethanol, 5 g/L of tartaric acid, 50 mg/L of each acetaldehyde, methanol, ethyl acetate, 1-propanol and isobutanol, and 150 mg/L of isoamyl alcohol. Working solutions were adjusted to pH=3.2 with 0.1 M NaOH.

Analysis of volatile aroma compounds by headspace solid-phase microextraction with gas chromatography/ mass spectrometry

Volatile aroma compounds were isolated using headspace solid-phase microextraction (HS-SPME) according to the modified method of Noguerol-Pato *et al.* (*38*), and analysed by gas chromatography/mass spectrometry (GC/MS). SPME fibre holder and 50/30 nm divinylbenzene-carboxen-polydimethylsiloxane (DVB-CAR-PDMS) fibres were purchased from Supelco (Bellefonte, PA, USA). Wine sample was diluted fourfold, and 4 mL of the solution were placed in a 10-mL glass vial. A volume of 50 µL of internal standard solution (0.84 mg of 2-octanol per L of wine for determination of terpenes, norisoprenoids, alcohols and miscellaneous compounds, 0.82 mg of methyl nonanoate per L of wine for determination of esters, and 2.57 mg of heptanoic acid per L of wine for determination of acids) and 1 g of ammonium sulphate were added. The vial was sealed with a Teflon-faced septum cap, and the sample was pre-conditioned at 40 °C for 15 min. Microextraction lasted for 40 min at 40 °C with stirring (800 rpm). For desorption, the fibre was inserted into the GC/MS injector port at 248 °C for 5 min (3 min in splitless mode).

Identification and quantification of minor volatile compounds was performed using a Varian 3900 gas chromatograph coupled with a Varian Saturn 2100T ion trap mass spectrometer (Varian Inc., Harbour City, CA, USA). The column used was an Rtx-WAX (60 m×0.25 mm i.d., 0.25 µm film thickness; Restek, Bellefonte, PA, USA). Initial oven temperature was 40 °C, then increased at 2 °C/ min to 240 °C, and then kept at 240 °C for 10 min. Injector, transfer line and ion trap temperatures were 245, 180 and 120 °C, respectively. Mass spectra were acquired in electron impact mode (70 eV) at 1 s/scan, full scan with a range of m/z=30–450. The carrier gas was helium (1 mL/ min). Identification was performed by comparing retention times and mass spectra with those of pure standards when available, and with mass spectra from NIST05 library (National Institute of Standards and Technology, Gaithersburg, MD, USA). Identification by comparison with mass spectra was considered successful for compounds with the MS spectra reverse match numbers higher than 800. Linear retention indices (relative to *n*-alkanes from C_{10} to C_{28}) were calculated and compared to those from literature (*22,39–44*). When standards were available, standard calibration curves were constructed. For other compounds semi-quantitative analysis was carried out, and their concentrations were expressed as equivalents of compounds with similar chemical structure for which standards were available, assuming a response factor equal to one: monoterpenes were quantified as linalool equivalents (response factor (RF) of linalool *vs.* 2-octa-

nol: 1.97), C_6 compounds as 1-hexanol (RF of 1-hexanol *vs.* 2-octanol: 0.14), alcohols as 2-octanol, fatty acids as heptanoic acid, ethyl and other esters as ethyl hexanoate (RF of ethyl hexanoate *vs.* methyl nonanoate: 1.71), acetate esters as hexyl acetate (RF of hexyl acetate *vs.* methyl nonanoate: 1.05), and miscellaneous compounds as 2-octanol equivalents.

Odour activity values and aroma compound groups

Odour activity values (OAV) of volatile aroma compounds were calculated as the quotients of their concentration and the corresponding odour perception threshold from literature (*45–48*). The OAVs of the compounds that exhibit similar olfactory sensation were grouped based on their odour description, as suggested by Moyano *et al.* (*49*). In this work eight groups (also known as series) were established: varietal terpenic, varietal fruity, sweet, fermentative fruity, berry fruit, floral, fatty and green. Such a presentation of wine aroma profile is an approximation and may differ from the results of sensory analysis performed by a panel of trained tasters. However, it is certainly valid for establishing the potential olfactory impact of particular groups and individual compounds. In addition, it greatly reduces the number of variables to be considered, and facilitates the interpretation of results (*49*).

Statistical analysis

All experiments were performed in duplicate, and average values were used in further data analysis. Mean values of concentration and standard deviations were calculated from five replicates, *i.e.* five samples of each investigated variety per harvest year. One- and two-way analyses of variance (ANOVA) and Fisher's least significant difference (LSD) test were used to compare the mean values at the level of significance of $p<0.05$. To differentiate wines according to varietal origin and harvest year, stepwise linear discriminant analysis (SLDA) was applied, using Wilks' lambda as a selection criterion and *F*-statistic factor to establish the significance of the changes in lambda when a new variable is tested. The prediction capacity of the discriminant model was estimated by cross--validation. Statistical elaboration was carried out using Statistica v. 8.0 software (StatSoft Inc., Tulsa, OK, USA).

Results and Discussion

The standard physicochemical parameters determined in wines made from Malvazija istarska, Chardonnay and Muscat yellow varieties in 2013 and 2014 are presented in Table 1. One-way analysis of variance (ANOVA) and Fisher's least significant difference (LSD) test were applied to compare the differences between the mean values for each harvest year separately. Two-way ANOVA with factor variety and harvest year was applied to establish if these differences were consistent in the two harvest years, as well as to determine the effect of harvest year. When two years were considered separately, no significant differences were observed, except for the higher concentration of reducing sugars in Muscat yellow group,

Table 1. Standard physicochemical parameters of Malvazija istarska, Chardonnay and Muscat yellow monovarietal wines produced in 2013 and 2014

| Physicochemical parameter | 1-way ANOVA | | | | | | 2-way ANOVA | | |
| | 2013 | | | 2014 | | | | | |
	Malvazija istarska	Chardonnay	Muscat yellow	Malvazija istarska	Chardonnay	Muscat yellow	V	Y	I
φ(alcohol)/%	13.0±0.3	12.8±0.5	12.1±0.7	12.2±0.3	12.5±0.5	12.0±1.1	n.s.	n.s.	n.s.
γ(reducing sugars)/(g/L)	(2.6±1.0)[b]	(3.4±1.2)[b]	(29.6±16.8)[a]	(2.5±0.4)[b]	(1.7±0.3)[b]	(27.0±9.0)[a]	*	n.s.	n.s.
γ(total dry extract without sugars)/(g/L)	19.3±0.8	20.3±0.9	20.3±1.6	21.5±0.7	21.5±0.9	22.9±1.3	n.s.	*	n.s.
γ(ash)/(g/L)	2.0±0.2	2.2±0.2	2.5±0.4	2.6±0.2	2.5±0.3	2.5±0.4	n.s.	*	n.s.
Total acidity/(g/L)	5.2±0.3	5.6±0.2	5.3±0.7	6.2±0.8	6.1±0.4	6.4±0.8	n.s.	*	n.s.
pH	3.29±0.07	3.3±0.1	3.4±0.2	3.4±0.1	3.5±0.1	3.4±0.1	n.s.	n.s.	n.s.
Volatile acidity/(g/L)	0.28±0.05	0.27±0.08	0.33±0.09	0.25±0.04	0.26±0.07	0.3±0.1	n.s.	n.s.	n.s.

Results are expressed as mean value±standard deviation, N=5. Different lowercase letters in superscript in a row represent statistically significant differences between mean values at p<0.05 obtained by one-way ANOVA and Fisher's least significant difference (LSD) test for each harvest year separately.

Two-way ANOVA factors: V=variety, Y=harvest year, I=interaction (V×Y); asterisk represents a statistically significant effect at p<0.05 by 2-way ANOVA; n.s.=not significant

which was expected since it consisted of dry, semi-dry, and semi-sweet wines. Two-way ANOVA revealed significant differences, with higher concentrations of extract and ash, and higher total acidity found in wines from 2014.

The concentrations of volatile aroma compounds determined by HS-SPME-GC/MS analysis in wines made from Malvazija istarska, Chardonnay and Muscat yellow varieties in 2013 and 2014 are presented in Table 2.

Varietal aroma compounds

Terpenic compounds originate from grapes, both as free volatile molecules and released from glycosidic precursors. As expected, Muscat yellow wines were the most abundant in terpenes, both qualitatively and quantitatively, with the domination of exceptionally high linalool concentration (Table 2). Such a composition is relatively in agreement with that previously determined in Muscat yellow must (*34,35*) and wine (*36*), where linalool concentrations were higher than 2500 µg/L in some cases. In this work, terpenic diols and particular other terpenes (hydroxyl forms) were not found probably because of their weaker volatility, which is also the reason why the concentration of linalool oxides was probably underestimated during semi-quantitative analysis relative to highly volatile linalool. On the other hand, the occurrence of many other monoterpenes in Muscat yellow wines, such as epoxylinalool, *trans*-β-ocimene, 6,10-dihydromyrcenol, menthol and *trans*-nerolidol has, to our knowledge, been confirmed for the first time.

In 2013, several monoterpenes, such as epoxylinalool, β-pinene, nerol oxide, and major monoterpenols linalool, hotrienol, α-terpineol, nerol and geraniol, were found in significantly higher concentrations in Malvazija istarska than in Chardonnay wines. Nerol emerged as a consistent differentiator of Malvazija and Chardonnay, with significant difference determined in both years. The concentrations and the composition of monoterpenes in Malvazija wines were generally in fair agreement with those reported previously: their content was moderate but significant, with linalool followed by geraniol as the most abundant (*9,22–25*). Although relatively high concentrations of linalool were found in particular Chardonnay wines from other world regions (up to 142 µg/L), wines from this variety are mostly deficient in monoterpenes (*30*), and the results of this study confirmed it.

The effect of variety was found to be significant for the majority of terpenes by two-way ANOVA, mostly due to significantly higher levels in Muscat wines (Table 2). The effect of year was also established for many terpenic compounds, with higher amounts generally found in wines produced in harvest 2013, which was characterised by more favourable climatic conditions. The response of each variety to climatic conditions of the two harvest years was different; the effect of year was more evident in Muscat and Malvazija wines, and less in Chardonnay. This was confirmed by significant interaction effects on several terpenes (Table 2). Interestingly, the effect of harvest year was not significant for the majority of the most important, major monoterpenols.

C_{13} norisoprenoid β-damascenone derives from the degradation of carotenoid molecules, such as β-carotene, lutein, neoxanthin and violaxanthin, during fermentation (*50*), and has an important positive role in wine varietal aroma because of its low odour perception threshold and pleasant odour reminiscent of honey, dried plum, and stewed apple. It was previously reported among the important contributors of both Malvazija istarska (*22,23*) and Chardonnay aroma (*26,30*). Significant differences between its content in the investigated monovarietal wines were not found. Such a result confirmed that the levels of β-damascenone are more dependent on viticultural and winemaking conditions than on variety (*50*).

C_6 compounds are mostly formed during prefermentation production steps by the enzymatic degradation of unsaturated fatty acids and from glycosidic precursors. They contribute to wine aroma with vegetal and herba-

Table 2. HS-SPME-GC/MS profiles of volatile aroma compounds in Malvazija istarska, Chardonnay and Muscat yellow monovarietal wines produced in 2013 and 2014

Volatile aroma compound	LRI	ID	RM	FM	1-way ANOVA						2-way ANOVA		
					2013			2014			V	Y	I
					Malvazija istarska	Chardonnay	Muscat yellow	Malvazija istarska	Chardonnay	Muscat yellow			
					γ(volatile compound)/(μg/L) or *(mg/L)								
Terpenes													
Epoxylinalool	1111	MS, LRI	802	721	(0.6±0.4)[b]	(0.1±0.1)[c]	(7.1±2.0)[a]	(0.2±0.2)[b]	(0.1±0.2)[b]	(3.2±0.8)[a]	**	**	**
β-Pinene	1145	MS, LRI	863	863	(2.8±1.1)[b]	(0.1±0.1)[c]	(41.9±17.0)[a]	(2.9±1.8)[b]	(0.7±0.9)[b]	(44.7±14.3)[a]	**	**	n.s.
Limonene	1196	MS, LRI	854	831	(2.5±0.1)[b]	(1.9±0.5)[b]	(27.3±4.5)[a]	(1.9±0.9)[b]	(1.2±0.7)[b]	(21.5±4.0)[a]	**	**	n.s.
β-Phellandrene	1203	MS, LRI	865	844	n.d.	n.d.	1.9±0.85	n.d.	n.d.	1.7±0.2	**	n.s.	n.s.
trans-β-Ocimene	1253	MS, LRI	812	793	n.d.	n.d.	11.7±4.43	(0.5±0.9)[b]	(0.1±0.2)[b]	(13.4±3.7)[a]	**	n.s.	n.s.
α-Terpinolene	1281	MS, LRI	894	864	(0.1±0.2)[b]	n.d.	(6.0±1.6)[a]	(0.3± 0.5)[b]	n.d.	(6.5±3.0)[a]	**	n.s.	n.s.
Monoterpene (n.i.)	1322	MS			n.d.	n.d.	14.2±5.4	n.d.	n.d.	10.4±3.9	**	n.s.	**
1,3,5-Tetramethyl-1,3-cyclohexadiene	1370	MS	865	801	n.d.	n.d.	3.4±1.3	n.d.	n.d.	3.0±0.9	**	n.s.	n.s.
trans-Linalool furan oxide	1436	MS, LRI	873	790	n.d.	n.d.	19.3±5.4	n.d.	n.d.	10.8±2.2	**	**	**
p-Menth-1-en-8-ol	1446	MS	807	734	n.d.	n.d.	16.8±3.0	n.d.	n.d.	7.4±3.2	**	**	**
6,10-Dihydromyrcenol	1457	MS, LRI	820	762	0.8±0.6	1.2±0.67	0.8±0.1	1.3±0.8	0.9±0.6	0.6±0.3	n.s.	n.s.	n.s.
Nerol oxide	1459	MS, LRI	828	795	(2.2±1.1)[b]	(0.5±0.4)[c]	(29.1±8.1)[a]	(1.2±0.9)[b]	(0.6±0.8)[b]	(11.7±3.7)[a]	**	**	**
cis-Linalool furan oxide	1464	MS, LRI	888	804	n.d.	(0.2±0.4)[b]	(9.2±3.3)[a]	(0.4±0.5)[b]	n.d.	(3.8±1.1)[a]	**	**	**
Geranyl ethyl ether 1	1499	MS, LRI	828	795	n.d.	n.d.	13.0±4.5	n.d.	n.d.	6.2±2.7	**	**	**
Monoterpene (n.i.)	1502	MS			(0.2±0.3)[b]	n.d.	(4.5±3.8)[a]	(0.1±0.1)[b]	(0.0±0.0)[b]	(1.5±0.8)[a]	**	n.s.	n.s.
Linalool	1542	S, MS, LRI	843	835	(54.1±21.3)[b]	(20.4±11.3)[c]	(670.5±259.1)[a]	(33.6±19.0)[b]	(18.9±7.8)[b]	(723.3±131.2)[a]	**	**	**
Isobornyl acetate	1587	MS, LRI	886	851	0.3±0.1	0.3±0.1	0.3±0.1	0.3±0.1	0.2±0.1	0.3±0.1	n.s.	n.s.	n.s.
Hotrienol	1601	MS, LRI	831	788	(4.7±2.1)[b]	(1.0±0.6)[c]	(90.6±25.7)[a]	(2.5±2.4)[b]	(1.2±1.0)[b]	(55.3±11.5)[a]	**	**	**
Menthol	1628	MS, LRI	914	814	3.4±1.3	2.4±1.3	1.7±0.8	(3.2±1.2)[a]	(2.5±1.6)[a]	(1.2±0.8)[b]	**	**	n.s.
α-Terpineol	1684	S, MS, LRI	850	830	(23.7±9.2)[b]	(7.9±4.4)[c]	(191.2±38.2)[a]	(10.8±7.3)[b]	(8.1±5.2)[b]	(124.5±38.6)[a]	**	**	**
α-Terpinenyl acetate	1688	MS, LRI	871	780	(0.9±0.4)[b]	(0.6±0.1)[b]	(3.5±0.5)[a]	(0.7±0.3)[b]	(0.6±0.4)[b]	(2.4±0.6)[a]	**	**	**
Geranyl acetate	1749	MS, LRI	878	816	n.d.	n.d.	n.d.	n.d.	n.d.	3.5±2.1	**	**	**
trans-Linalool pyran oxide	1752	MS, LRI	809	800	(0.1±0.2)[b]	n.d.	(10.2±3.4)[a]	(0.6±0.8)[b]	n.d.	(5.5±2.1)[a]	**	**	**
Citronellol	1758	S, MS, LRI	864	778	(4.0±0.8)[b]	(3.5±0.7)[b]	(23.2±13.2)[a]	(3.7±1.6)[b]	(2.9±0.5)[b]	(31.9±14.4)[a]	**	n.s.	n.s.
Nerol	1791	S, MS, LRI	929	807	(5.7±2.8)[b]	(0.9±1.1)[c]	(70.7±24.4)[a]	(4.0±2.0)[b]	(0.7±0.5)[c]	(76.3±15.8)[a]	**	n.s.	n.s.
Geraniol	1838	S, MS, LRI	902	811	(23.2±5.2)[b]	(14.0±3.2)[c]	(196.5±45.3)[a]	(16.4±8.2)[b]	(12.747±3.4)[b]	(170.0±45.7)[a]	**	n.s.	n.s.
Geranyl acetone	1845	MS, LRI	885	811	(0.8±0.5)[b]	(1.1±0.4)[b]	(3.2±1.2)[a]	(1.2±0.9)[b]	(1.1±1.0)[b]	(2.9±1.4)[a]	**	n.s.	n.s.

Table 2. – continued

Volatile aroma compound	LRI	ID	RM	FM	1-way ANOVA						2-way ANOVA		
					2013			2014			V	Y	I
					Malvazija istarska	Chardonnay	Muscat yellow	Malvazija istarska	Chardonnay	Muscat yellow			
					γ(volatile compound)/(μg/L) or *(mg/L)								
trans-Nerolidol	2031	MS, LRI	874	804	1.4±0.3	1.8±0.8	2.0±1.0	0.89±0.3	1.0±0.3	1.3±0.3	n.s.	**	n.s.
Geranic acid	2319	MS, LRI	838	749	n.d.	n.d.	4.7±1.8	n.d.	n.d.	7.0±2.2	**	n.s.	n.s.
Farnesol	2341	MS, LRI	907	799	0.3±0.2	0.6±0.3	0.7±0.5	(0.4±0.1)[b]	(0.6±0.1)[ab]	(0.7±0.2)[a]	n.s.	n.s.	n.s.
Total terpenes					(130.8±39.6)[b]	(58.0±21.8)[c]	(1474.7±384.5)[a]	(86.7±43.6)[b]	(54.1±18.9)[b]	(1352.3±197.3)[a]	**	n.s.	n.s.
C₁₃ norisoprenoids													
β-damascenone	1809	S, MS, LRI	853	830	1.1±0.3	1.4±0.3	1.0±0.5	1.3±0.4	1.3±0.3	1.2±0.6	n.s.	n.s.	n.s.
Norisoprenoid (n.i.)	2119	MS			0.9±0.4	1.3±0.4	0.8±0.5	1.0±0.4	0.8±0.1	1.8±1.7	n.s.	n.s.	n.s.
Total C₁₃ norisoprenoids					(1.9±0.7)[ab]	(2.7±0.5)[a]	(1.8±0.6)[b]	2.3±0.7	2.1±0.3	3.0±1.2	n.s.	n.s.	n.s.
C₆ compounds													
Hexanal	1080	MS, LRI	889	823	1.7±0.4	2.4±0.7	4.2±4.1	2.4±1.2	3.1±1.3	3.3±1.7	n.s.	n.s.	n.s.
cis-3-Hexen-1-yl acetate	1316	MS, LRI	826	803	(116.3±65.9)[b]	(152.3±43.2)[ab]	(277.4±160.5)[a]	78.8±48.3	78.7±17.5	210.3±154.0	**	n.s.	n.s.
1-Hexanol*	1356	S, MS, LRI	876	817	1.3±0.4	1.1±0.1	1.1±0.2	(1.4±0.3)[a]	(1.3±0.3)[ab]	(1.0±0.1)[b]	**	n.s.	n.s.
trans-3-Hexen-1-ol	1361	MS, LRI	861	839	(41.6±12.9)[a]	(32.1±13.8)[ab]	(15.5±4.8)[b]	33.9±14.1	31.1±11.7	27.8±15.7	n.s.	n.s.	n.s.
cis-3-Hexen-1-ol	1379	MS, LRI	871	844	(51.6±13.2)[b]	(38.4±2.4)[b]	(141.4±52.0)[a]	(34.3±10.0)[b]	(32.0±10.4)[b]	(87.8±33.6)[a]	**	**	n.s.
Total C₆ compounds*					1.5±0.4	1.3±0.2	1.5±0.2	1.6±0.3	1.4±0.3	1.3±0.3	n.s.	n.s.	n.s.
Alcohols													
2-Ethyl-1-hexanol	1485	MS, LRI	851	835	11.4±3.3	9.3±3.9	17.8±15.2	9.1±3.7	11.8±7.1	7.3±2.1	n.s.	n.s.	n.s.
1-Octanol	1552	MS, LRI	887	841	(19.9±2.3)[a]	(16.9±4.7)[ab]	(14.8±2.3)[b]	16.5±2.5	16.7±1.5	15.0±3.8	n.s.	n.s.	n.s.
2-Phenylethanol*	1893	S, MS, LRI	956	956	21.7±5.6	16.1±2.2	16.5±6.5	13.5±2.6	17.1±5.1	13.6±1.3	**	**	n.s.
Total alcohols*					21.7±5.6	16.1±2.2	16.6±6.5	13.5±2.6	17.1±5.1	13.6±1.4	**	n.s.	n.s.
Fatty acids													
Hexanoic acid*	1830	S, MS, LRI	813	738	(12.0±1.7)[a]	(12.9±2.1)[a]	(8.5±2.7)[b]	8.6±2.1	9.0±3.1	9.7±2.7	n.s.	n.s.	n.s.
Octanoic acid*	2043	S, MS, LRI	889	759	11.6±1.2	11.3±3.2	9.9±3.6	7.4±2.4	7.9±2.5	12.0±4.8	n.s.	n.s.	n.s.
Nonanoic acid*	2135	MS, LRI	831	802	(0.3±0.1)[ab]	(0.8±0.6)[a]	(0.2±0.1)[b]	(1.1±0.3)[a]	(1.4±0.7)[a]	(0.3±0.1)[b]	**	**	n.s.
Decanoic acid*	2257	S, MS, LRI	820	805	2.1±0.7	1.7±0.7	3.2±1.5	(0.8±0.3)[b]	(1.0±0.3)[b]	(4.0±2.1)[a]	**	n.s.	n.s.
Dodecanoic acid	2467	MS, LRI	869	865	125.3±42.0	101.7±40.6	127.7±68.2	64.2±13.0	109.4±27.8	126.7±79.4	n.s.	n.s.	n.s.
Total fatty acids*					26.1±3.1	26.7±5.9	21.9±7.7	18.0±4.4	19.5±5.5	26.1±9.4	n.s.	n.s.	n.s.

Table 2. – continued

Volatile aroma compound	LRI	ID	RM	FM	1-way ANOVA						2-way ANOVA		
					2013			2014			V	Y	I
					Malvazija istarska	Chardonnay	Muscat yellow	Malvazija istarska	Chardonnay	Muscat yellow			
					γ(volatile compound)/(μg/L) or *(mg/L)								
Ethyl esters													
Ethyl propionate	<1000	MS, LRI	835	760	1.6±0.2	2.0±0.6	1.7±0.3	1.5±0.3	1.5±0.3	1.2±0.2	n.s.	n.s.	n.s.
Ethyl isobutyrate	<1000	MS, LRI	811	721	(33.6±9.0)[a]	(17.1±5.6)[b]	(24.2±4.7)[ab]	16.8±9.6	18.6±8.2	17.2±6.7	n.s.	**	n.s.
Ethyl butyrate*	1030	S, MS, LRI	859	766	0.5±0.1	0.5±0.1	0.5±0.1	0.45±0.07	0.5±0.1	0.5±0.1	n.s.	n.s.	n.s.
Ethyl 2-methylbutyrate	1049	S, MS, LRI	851	800	(8.0±2.8)[a]	(4.0±1.5)[b]	(3.4±1.0)[b]	3.3±1.0	3.7±1.6	2.0±0.9	**	*	**
Ethyl 3-methylbutyrate	1065	S, MS, LRI	800	722	(19.4±6.1)[a]	(9.3±3.6)[b]	(8.8±1.0)[b]	9.2±1.9	8.2±4.8	5.2±3.0	**	**	n.s.
Ethyl 2-butenoate	1161	MS, LRI	852	844	2.0±1.0	2.1±0.3	1.5±0.7	2.8±1.1	2.2±0.5	2.2±1.1	n.s.	n.s.	n.s.
Ethyl hexanoate*	1236	S, MS, LRI	868	826	0.6±0.1	0.8±0.1	0.7±0.2	(0.4±0.2)[b]	(0.5±0.2)[b]	(0.8±0.1)[a]	**	n.s.	**
Ethyl heptanoate	1332	MS, LRI	849	804	(11.2±3.5)[b]	(20.9±7.7)[a]	(8.4±3.3)[b]	(27.2±8.7)[ab]	(32.9±8.3)[a]	(16.5±7.2)[b]	**	**	n.s.
Ethyl octanoate*	1435	S, MS, LRI	862	784	(1.1±0.3)[b]	(1.8±0.4)[a]	(1.3±0.9)[a]	(1.0±0.3)[b]	(1.3±0.6)[ab]	(1.8±0.4)[a]	**	**	n.s.
Ethyl nonanoate	1534	MS, LRI	820	786	(18.3±8.1)[ab]	(34.2±17.6)[a]	(10.4±5.2)[b]	(32.4±9.5)[ab]	(46.5±19.5)[a]	(16.1±2.6)[b]	**	**	n.s.
Ethyl decanoate*	1637	S, MS, LRI	830	770	0.39±0.05	0.4±0.1	0.7±0.4	(0.5±0.2)[ab]	(0.36±0.08)[b]	(0.6±0.2)[a]	**	n.s.	n.s.
Ethyl dodecanoate	1842	MS, LRI	769	769	9.9±0.7	12.0±2.5	12.1±6.8	9.4±1.8	12.8±1.5	12.3±3.3	n.s.	n.s.	n.s.
Ethyl tetradecanoate	2045	MS, LRI	803	800	7.4±0.7	8.0±1.5	6.0±2.4	5.9±1.8	6.91±1.5	7.9±2.5	n.s.	n.s.	n.s.
Total ethyl esters*					2.6±0.5	3.8±0.4	3.2±1.5	2.5±0.6	2.8±0.9	3.3±1.2	n.s.	n.s.	n.s.
Acetate esters													
Methyl acetate	<1000	MS, LRI	888	861	2.5±0.7	2.6±0.7	2.4±0.8	3.7±1.0	3.0±1.0	3.6±2.1	n.s.	n.s.	n.s.
Isobutyl acetate	1009	S, MS, LRI	842	734	(58.0±18.2)[b]	(111.0±13.7)[a]	(85.0±28.5)[ab]	97.3±38.4	132.4±17.2	133.0±17.7	**	**	n.s.
Butyl acetate	1072	MS, LRI	868	807	(3.4±1.9)[b]	(7.3±1.8)[a]	(4.6±3.1)[ab]	(4.9±1.6)[b]	(9.6±4.4)[a]	(4.5±2.4)[b]	**	n.s.	n.s.
Isoamyl acetate*	1120	S, MS, LRI	885	839	3.2±1.2	5.4±0.8	4.3±2.5	4.0±1.6	4.6±0.7	5.2±2.3	n.s.	n.s.	n.s.
Hexyl acetate*	1272	S, MS, LRI	905	822	0.15±0.04	0.23±0.06	0.2±0.1	0.18±0.08	0.17±0.04	0.3±0.1	n.s.	n.s.	n.s.
2-Phenylethyl acetate*	1803	S, MS, LRI	856	856	(2.9±0.8)[b]	(4.6±0.5)[a]	(3.0±0.9)[b]	2.1±0.5	3.6±0.7	3.4±1.5	**	n.s.	n.s.
Total acetate esters *					(6.3±1.8)[b]	(10.7±1.3)[a]	(7.6±3.3)[ab]	6.4±1.8	8.4±0.6	9.0±3.9	n.s.	n.s.	n.s.
Other esters													
Methyl hexanoate	1192	MS, LRI	876	844	1.2±0.4	1.3±0.6	0.9±0.3	1.1±0.4	0.7±0.3	1.2±0.4	n.s.	n.s.	n.s.
Isoamyl formate	1196	MS	819	751	0.7±1.0	0.9±1.4	0.4±0.2	0.2±0.1	0.7±0.6	0.4±0.1	n.s.	n.s.	n.s.
Methyl octanoate	1387	MS, LRI	871	823	18.6±2.2	18.7±1.3	15.7±2.8	17.4±2.6	17.2±3.1	20.8±6.2	n.s.	n.s.	n.s.

Table 2. – continued

Volatile aroma compound	LRI	ID	RM	FM	1-way ANOVA						2-way ANOVA		
					2013			2014			V	Y	I
					Malvazija istarska	Chardonnay	Muscat yellow	Malvazija istarska	Chardonnay	Muscat yellow			
					γ(volatile compound)/(μg/L) or *(mg/L)								
Isoamyl hexanoate	1457	MS, LRI	835	835	(0.7±0.3)[b]	(1.5±0.4)[ab]	(2.0±0.8)[a]	1.3±0.6	1.4±0.4	1.6±0.4	**	n.s.	n.s.
Isoamyl heptanoate	1560	MS	822	798	0.9±0.8	1.5±1.3	1.0±0.7	1.2±0.6	1.2±0.5	0.6±0.5	n.s.	n.s.	n.s.
Isoamyl octanoate	1655	MS, LRI	800	722	6.4±2.1	6.2±1.6	4.8±1.9	5.7±1.2	7.3±1.0	8.3±2.6	n.s.	n.s.	n.s.
Diethyl succinate*	1667	S, MS, LRI	859	827	(2.2±0.9)[a]	(1.7±0.8)[ab]	(0.9±0.3)[b]	1.1±0.6	1.20±0.1	0.9±1.1	n.s.	n.s.	n.s.
Ester (n.i.)	1710	MS			(3.4±1.0)[b]	(4.9±0.4)[a]	(6.1±1.2)[a]	(2.5±0.4)[b]	(3.4±1.0)[ab]	(5.0±1.8)[a]	**	**	**
Methyl salicylate	1759	MS, LRI	887	852	(0.9±0.5)[a]	(0.24±0.07)[b]	(0.09±0.03)[b]	0.5±0.1	0.5±0.3	0.4±0.1	**	n.s.	n.s.
Isoamyl decanoate	1862	MS, LRI	800	748	1.0±0.2	0.8±0.4	0.7±0.4	(3.6±3.1)[a]	(0.6±0.2)[b]	(0.9±0.4)[ab]	**	n.s.	n.s.
Ethyl cinnamate	2111	S, MS, LRI	848	790	(1.4±0.3)[b]	(3.2±1.0)[a]	n.d.	(1.0±0.3)[b]	(2.3±0.5)[a]	(0.8±1.0)[b]	**	n.s.	**
Hexyl salicylate	2203	MS, LRI	801	771	1.0±0.1	1.0±0.2	1.3±0.3	0.8±0.3	0.7±0.2	1.0±0.2	**	**	n.s.
Total other esters*					(2.2±1.0)[a]	(1.8±0.8)[ab]	(0.9±0.3)[b]	1.2±0.6	1.24 ± 0.09	0.9±1.1	n.s.	n.s.	n.s.
Miscellaneous													
2-Heptanone	1182	MS, LRI	875	800	1.5±0.7	1.2±1.1	1.3±1.2	(0.9±0.4)[ab]	(0.8±0.2)[b]	(1.4±0.3)[a]	n.s.	n.s.	n.s.
Benzaldehyde	1508	S, MS, LRI	864	864	(4.2±1.1)[b]	(9.5±1.7)[a]	(2.5±0.7)[c]	10.1±6.8	12.7±4.9	10.8±4.8	n.s.	**	n.s.
Ethyl benzeneacetate	1773	MS, LRI	914	858	1.2±0.4	1.3±0.5	1.4±1.2	2.2±1.5	2.2±0.8	2.6±1.7	n.s.	**	n.s.
Dimethylbenzaldehyde isomer	1790	MS	873	873	(17.8±2.5)[b]	(24.9±4.1)[a]	(2.4±1.1)[c]	(7.4±5.4)[b]	(13.4±3.0)[a]	(2.8±2.0)[b]	**	**	**
γ-Nonalactone	2008	MS, LRI	839	763	0.9±0.5	0.7±0.2	1.1±0.5	1.5±0.9	1.0±0.2	2.4±1.3	**	**	n.s.
4'-Ethoxy-2'-hydroxyoctanophenone	2090	MS, LRI	759	724	2.7±0.6	3.1±0.5	3.7±1.1	(2.1±0.7)[b]	(1.8±0.6)[b]	(4.0±1.9)[a]	**	**	n.s.
4-Vinylguaiacol	2175	MS, LRI	903	903	7.2±1.5	6.3±6.6	8.1±4.0	5.1±2.3	6.8±2.6	8.1±3.8	n.s.	n.s.	n.s.
2-(Phenylmethylene)-octanal	2356	MS, LRI	839	832	4.2±1.3	3.4±1.3	4.0±0.9	(2.5±1.2)[b]	(2.1±1.8)[b]	(4.4±1.0)[a]	**	n.s.	**
1,2-Benzenedimethanol	2376	MS	853	822	2.5±1.3	5.9±6.1	2.0±0.8	1.6±0.7	5.4±2.5	5.9±4.8	n.s.	n.s.	n.s.
Total miscellaneous					(42.5±5.1)[b]	(56.3±13.3)[a]	(26.9±5.2)[c]	33.6±11.0	46.5±3.8	42.9±12.2	**	n.s.	**

LRI=linear retention index; ID=identification of compounds: S=retention time and mass spectrum consistent with that of the pure standard and with NIST05 mass spectra electronic library, MS=mass spectra consistent with those from NIST05 mass spectra electronic library, LRI=linear retention index consistent with that found in literature (*39–44*). The compounds for which pure standards were not available (without letter S in the ID column) were quantified semi-quantitatively, and their concentrations were expressed as equivalents of compounds with similar chemical structure assuming a response factor=1. The compounds with only MS symbol in the ID column were tentatively identified. RM=mass spectra reverse match number; FM=mass spectra forward match number; n.i.=not identified; n.d.=not detected.

Different lowercase superscript letters in a row represent statistically significant differences between mean values at p<0.05 obtained by one-way ANOVA and Fisher's least significant difference (LSD) test for each harvest year separately.

Two-way ANOVA factors: V=variety, Y=harvest year, I=interaction (V×Y); **=statistically significant effect at p<0.05 by 2-way ANOVA; n.s.=not significant

ceous odours, and may have a negative effect when present in high concentration. Some authors consider C_6 compounds to be varietal aromas (*13*), and their ratios were shown to be useful for varietal differentiation of certain wines (*19*). In this work, significantly higher concentrations of *cis*-3-hexenyl derivatives (in both years) and lower concentrations of *trans*-3-hexen-1-ol and 1-hexanol (in 2013 and 2014, respectively) were noted in Muscat yellow than in other investigated monovarietal wines (Table 2).

Other compounds originating from grapes, polyfunctional thiols, have also been identified in Chardonnay wines in earlier investigations, but their impact was not found to be as important as in the case of Sauvignon blanc, where they are crucial for typical varietal aroma (*30*). Analysis of thiols is not a trivial task because of their very low abundance and reactivity, and this may account for the fact that their occurrence in Malvazija istarska and Muscat yellow has not been confirmed up to date. Although thiols were targeted compounds in this work (standards were available), they were not identified in any of the wines by the HS-SPME-GC/MS method.

Fermentation aroma compounds

Concentrations and the composition of wine major aroma compounds produced during fermentation, such as alcohols, straight-chain acids, and ethyl and acetate esters, were in a fair agreement with those found in the three varieties investigated earlier (*22,23,30,31,51*), and it was confirmed that they make up a basis of the aroma profile of unoaked young white wines. They depend mostly on fermentation parameters and conditions, although physicochemical composition of must may have a certain influence (*52*). Several studies reported that fermentation aroma compound composition significantly depends on varietal origin and harvest year, and can be useful in differentiating wines on the basis of these two criteria (*7,37*). This especially refers to medium-chain fatty acids and their ethyl esters which, unlike acetates, depend more on the availability of substrates, and therefore agricultural conditions and variety, than on enzymatic activity of yeast (*31*). For example, it was shown that South African unoaked young Chardonnay wines can be discriminated from other varieties (correct classification of 74 %) on the basis of 2-phenylethanol, diethyl succinate, ethyl hexanoate, ethyl decanoate, ethyl octanoate, hexyl acetate and 1-propanol concentrations (*31*).

The concentrations of major straight-chain acids (C6, C8 and C10) were notable in all wines, surpassing their odour perception thresholds of 420, 500 and 1000 μg/L, respectively (*45*). Fatty acid production is determined in part by the initial composition of must (*53*). Significant differences among varieties were found in hexanoic and decanoic acid, but in different harvests, implying the effect of harvest year. Low levels of nonanoic acid emerged as a potentially typical feature of Muscat yellow wines (Table 2). The effect of harvest year (two-way ANOVA) was also significant for this acid, with higher concentrations found in wines from 2014.

In 2013, Malvazija istarska wines stood out with higher concentrations of short, branched-chain ethyl esters, but contained lower ethyl octanoate concentration than other

investigated wines. In 2014, Muscat yellow wines had the highest concentration of major middle-chain ethyl esters (hexanoate, octanoate and decanoate). Ethyl esters with odd number of carbon atoms (heptanoate and nonanoate) were found useful in differentiating Chardonnay from Muscat yellow wines, being more abundant in the former. Two-way ANOVA results showed a significant effect of variety on the concentrations of major straight-chain ethyl esters, while the effect of harvest year was observed on the majority of branched-chain ethyl esters, with higher concentrations noted in 2013. These compounds, together with higher alcohol acetates, derive mainly from the yeast amino acid metabolism, and it is well known that the concentration of amino acids in grape depends on climatic conditions (*7*). The effect of year was also significant on odd-chain ethyl esters, with significantly higher concentrations in wines from the less favourable harvest of 2014. Odd-chain ethyl esters basically followed the same pattern observed for the odd-chain fatty acids (Table 2).

Chardonnay wines were more abundant in acetate esters, with significant differences in some cases, more pronounced in 2013. In both harvest years, significant differences were found in a few other esters, in some cases corroborated by two-way ANOVA results. Ethyl cinnamate emerged as a consistent differentiator of Malvazija istarska and Chardonnay wines, with higher concentration in the latter. Such a result partly confirmed previous findings in which ethyl cinnamate was among the key compounds responsible for the typical aroma of Chardonnay (*26,27*). It is worth mentioning that the chromatographic peak of ethyl cinnamate in Muscat yellow wines interfered with a much larger signal belonging to an unknown compound, which obstructed its identification and quantification in the majority of samples.

Among other compounds, particular benzenoids were found to be characteristic for Chardonnay, especially in 2013 when they were able to differentiate all three wines according to varietal origin. Like ethyl cinnamate, a tentatively identified dimethylbenzaldehyde isomer, with mass spectra showing a rather high degree of similarity with that of 2,4-dimethylbenzaldehyde (characteristic ions with *m/z* (relative intensity): 133 (100), 134 (41), 105 (35), 77 (16), 75 (15), with mass spectra forward and reverse match number of 873), for which the standard was available, turned out to be a potentially consistent differentiator of Chardonnay wines in both years. Muscat yellow wines had higher levels of tentatively identified 4'-ethoxy-2'-hydroxy-octanophenone and 2-(phenylmethylene)-octanal in 2014. A significant effect of year was observed in the case of benzaldehyde, ethyl benzeneacetate, and γ-nonalactone with higher amounts found in wines from the rainy 2014. On the other hand, Malvazija istarska and Chardonnay wines from 2013 contained more dimethylbenzaldehyde than in 2014.

Although particular volatile compounds, such as linalool (*26,27,29*), α-terpineol (*26*), β-damascenone (*26*), hexanoic (*26*), octanoic (*26*), and decanoic acid (*26,29*), ethyl butyrate (*26,27*), ethyl 2-methylbutyrate (*26,27*), ethyl hexanoate (*26,27*), isoamyl acetate (*26,29*), 2-phenethyl acetate (*26*), diethyl succinate (*31*) and 4-vinylguaiacol (*26–28*), had previously been counted among the compounds important for unoaked Chardonnay wine typicity (*28*), in-

cluded among the key Chardonnay volatiles (*26*) and found to be decisive for the reconstitution of Chardonnay typical aroma (*29*), the results of this investigation showed that their amounts are probably not Chardonnay-specific, since they were not found useful as differentiators from Malvazija and/or Muscat yellow wines (Table 2).

Stepwise linear discriminant analysis

Aromatic Muscat yellow wines were clearly differentiated from relatively neutral Malvazija istarska and Chardonnay by (mono)terpene concentrations higher by an order of magnitude (Table 2). For this reason, further investigation was focused on determining the difference between the latter two, and stepwise linear discriminant analysis (SLDA) was applied only on the Malvazija istarska and Chardonnay data set. The number of groups was four, since wines from the same variety from different harvest year were considered as separate groups. SLDA model extracted 12 compounds according to Wilks' lambda criterion, and was successful in classifying wines according to both varietal origin and harvest year. A 100 % correct classification was achieved after the inclusion of only four compounds in the following order: dimethyl-benzaldehyde, isobutyl acetate, β-pinene, and *trans*-3--hexen-1-ol. The projection of monovarietal Malvazija istarska and Chardonnay wine samples classified according to variety and harvest year in two-dimensional space defined by the first two discriminant functions, as well as the compounds included in the model are shown in Fig. 1. The samples were grouped according to variety along the direction of the second, and according to harvest year along the direction of the first discriminant function. The prediction capacity of the SLDA model was evaluated by 'leave one-out' cross-validation, where each wine was removed from the model and classified by the functions derived from all cases other than that case. The percentage of correct prediction by cross-validation was also 100 %.

When SLDA was applied to the set of Malvazija istarska and Chardonnay samples divided into two groups based on variety, the obtained model included 16 compounds in total. Ethyl cinnamate entered the model as the first, emerging as the most potent differentiator, and classified correctly all Malvazija wines. The inclusion of butyl

acetate and 1,2-benzenedimethanol resulted in a 100 % correct classification of all wines. When two groups were formed with harvest year as a criterion, only two compounds were sufficient for a 100 % correct classification: dimethylbenzaldehyde and isobutyl acetate. Another 13 compounds entered and additionally improved the differentiation capacity of the model.

Impact odourants and aroma groups

One- and two-way ANOVA followed by SLDA analysis extracted many possible markers of varietal origin of Malvazija istarska, Chardonnay and Muscat yellow wines among the analysed volatile compounds. The differentiation was rather clear when considering Muscat wines, clearly distinguished from others by high concentrations of odouriferous monoterpenols (Table 2), which significantly surpassed the corresponding odour perception thresholds in the majority of samples from both years (linalool threshold 6, citronellol 18, nerol 15 and geraniol 30 μg/L) (*46–48*). It can be stated with certitude that the aroma of Muscat yellow wines was typically muscat. To explain the differences between aroma profiles of Malvazija istarska and Chardonnay, two typologically similar wines, represented a more challenging task. Many of the compounds found to discriminate those wines in this work are of doubtful or unknown sensory impact. In an attempt to approximate how the differences in chemical composition between Malvazija istarska and Chardonnay wines possibly reflect on their sensory quality, the quantified main odourants occurring in peri- and suprathreshold concentrations, and therefore exhibiting odour activity values (OAV) near and higher than 1, were selected and grouped into main aroma groups based on the similarity of their odours. Selected odourants are listed in Table 3 together with their odour descriptors and affiliation to corresponding aroma groups, while the constructed aroma group profiles are presented in Fig. 2.

The dominance of fruity aroma compounds formed in fermentation with ethyl octanoate as the most powerful odourant was determined in all wines (Fig. 2), and corresponded to a profile of a standard young white wine and previous findings on Malvazija and Chardonnay (*22,23,51*). Many of the esters formed in fermentation contributed to the formation of sweet aroma group, which
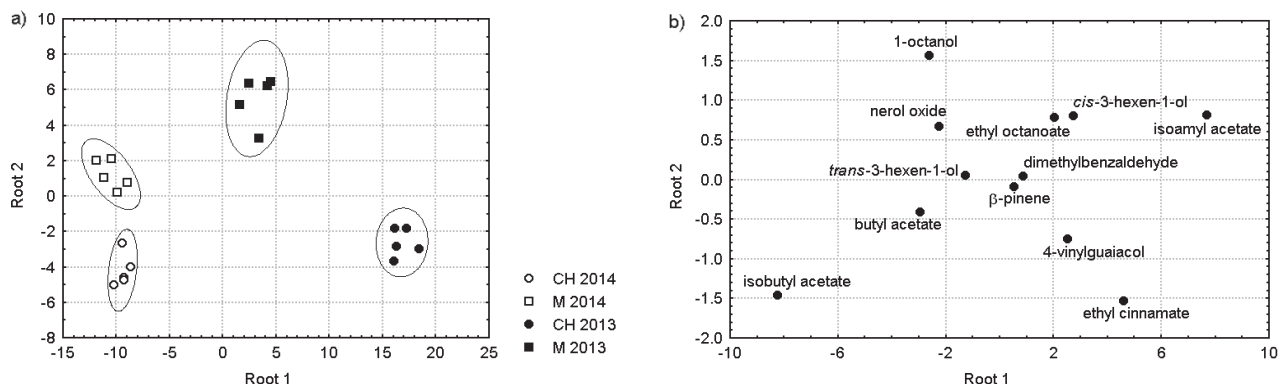
Fig. 1. Projection of Malvazija istarska (M) and Chardonnay (CH) monovarietal wines produced in 2013 and 2014, classified according to varietal origin and harvest year, along the directions of two discriminant functions by stepwise linear discriminant analysis (SLDA) (a), and standardised coefficients of compounds selected by the SLDA model (b)

Table 3. Odour perception thresholds (OPT) and odour descriptors (*45–48*), and aroma group affiliation of the main odourants found in Malvazija istarska and Chardonnay monovarietal wines produced in 2013 and 2014

Odourant	OPT/(µg/L)	Odour descriptor	Aroma group
Linalool	6	floral	varietal terpenic, floral
Citronellol	18	citrus	varietal terpenic, varietal fruity
Nerol	15	orange flowers, rose	varietal terpenic, floral
Geraniol	30	roses, geranium	varietal terpenic, floral
β-Damascenone	0.05	sweet, stewed apple, plum	varietal fruity, sweet
1-Hexanol	1620	fresh cut grass	green
cis-3-Hexen-1-ol	70	grass, herbaceous	green
2-Phenylethanol	10000	rose, talc, honey	floral
Hexanoic acid	420	cheese, rancid	fatty
Octanoic acid	500	cheese, rancid, fat	fatty
Decanoic acid	1000	rancid, waxen, plasticine	fatty
Ethyl isobutyrate	15	berry, blackberry	sweet, berry fruit
Ethyl butyrate	20	fruity	fermentative fruity
Ethyl 2-methylbutyrate	1	sweet fruit	sweet, berry fruit
Ethyl 3-methylbutyrate	3	berry, blackberry	sweet, berry fruit
Ethyl hexanoate	5	green apple	fermentative fruity
Ethyl octanoate	2	sweet, banana, pineapple	sweet, fermentative fruity
Ethyl decanoate	200	grape, fruit	fermentative fruity
Isoamyl acetate	30	banana	fermentative fruity
2-Phenethyl acetate	250	fruity, honey, floral	fermentative fruity, floral
Ethyl cinnamate	1	fruity, honey, cinnamon	sweet, fermentative fruity
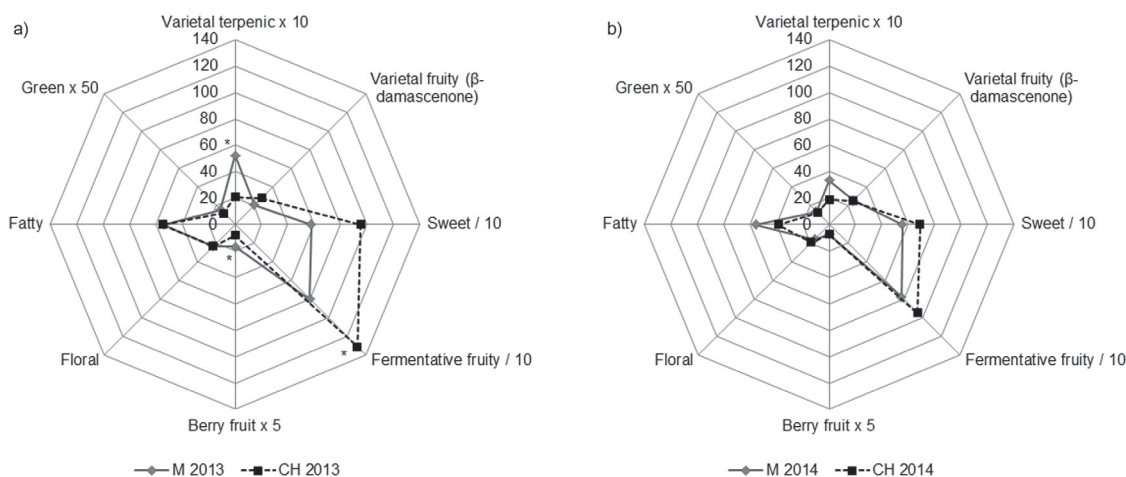
Fig. 2. Aroma profiles of Malvazija istarska (M) and Chardonnay (CH) monovarietal wines produced in 2013 (a) and 2014 (b), obtained on the basis of aroma group values calculated from the odour activity values of the corresponding aroma compounds. Asterisks denote statistically significant differences at p<0.05 obtained by one-way ANOVA and Fisher's least significant difference (LSD). Values of particular aroma group were multiplied or divided by a factor in order to obtain a more uniform display

was also among the dominant aromas. In Chardonnay wines, these two groups had higher values, suggesting that Chardonnay wine aroma was characterised by higher intensities of fruity and sweet odours than that of Malvazija. On the other hand, the profile of Malvazija istarska wines was somewhat more complex, with higher levels of the varietal terpenic group in both years, berry fruit group in 2013, and fatty group in 2014. It is possible that the sub-

tle, so-called 'muscat character' of Malvazija istarska wines, which is often encountered in practice, derives from the sensory activity of linalool supported by other major monoterpenols through synergistic and additive effects. The effect of harvest year was also evident: varietal terpenic, fruity and sweet groups prevailed in wines from 2013 in relation to those from 2014, implying the former were more aromatic.

Conclusions

By using volatile aroma profiles obtained by HS--SPME-GC/MS analysis, elaborated by univariate and multivariate statistics, a detailed characterisation of three monovarietal wines, Malvazija istarska, Chardonnay and Muscat yellow produced in Istria (Croatia) was achieved. Varietal differentiation of wines produced in climatically more favourable harvest of 2013 was more successful, and many compounds emerged as potential discriminators. Colder and humid 2014 exerted a significant effect by partially or totally annulling their differentiating ability, but extracted new markers specific for 2014. It is clear that variety and harvest year did not affect the variability in wine composition independently, but interacted with each other. Nevertheless, particular compounds emerged as statistically consistent markers of varietal origin through both vintages: Malvazija had specific nerol concentration, ethyl cinnamate and a dimethylbenzaldehyde isomer were characteristic for Chardonnay, while Muscat yellow wines were clearly discriminated by the highest concentration of terpenes. It is worth emphasising that several neglected compounds with small or no sensory significance, commonly not listed among wine origin differentiators, such as particular odd-chain acids and esters and benzenoids, were found to have interesting discrimination capacity. Apart from that, it was shown that not only varietal aromas, but several fermentation aroma compounds were significantly affected by variety. Wines from the more favourable harvest of 2013 contained higher concentrations of the majority of important volatile compounds than in 2014. A special attention was given to the comparison of Malvazija istarska and Chardonnay wines, two wines similar by typology, the former being a domestic, and the latter an introduced variety in Istria and Croatia. Malvazija wines were more abundant in monoterpenes, while Chardonnay aroma was characterised by higher concentrations of fruity esters. Such differences were pronounced and statistically significant in wines from 2013, but were partially overpowered by the effect of unfavourable 2014. Despite that, a 100 % correct classification of Malvazija istarska and Chardonnay wines according to both variety and harvest year was achieved by stepwise linear discriminant analysis, confirming that detailed HS-SPME-GC/MS aroma profiling accompanied by multivariate statistics is a powerful tool for differentiation of wines based on various criteria. The concept of odour activity values and aroma groups pointed to the potential differences between sensory profiles of Malvazija istarska and Chardonnay wines.

It is worth emphasising that this study represents the first successful attempt to compare and differentiate Malvazija istarska from other monovarietal white wines, as an important step in determination of its typicity and uniqueness. The study showed that many compounds which had been linked to typical Chardonnay aroma in earlier works have not been confirmed to be Chardonnay--specific, which confirms the importance of varietal differentiation and discrimination analysis, in order to determine the unique varietal characteristics of wines.

It was demonstrated that wine from Malvazija istarska variety is an interesting and viable alternative to common globally known varieties, such as Chardonnay, thus increasing the offer for consumers, and favouring differentiation of Croatian wines on the national and international market. The results obtained are of interest to the industry, and represent useful guidelines for the characterisation and differentiation of Istrian and Croatian monovarietal wines. However, further study is needed, comprising larger sample sets from several vintages. Such an investigation is currently being performed under the framework of a national scientific project.

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