Characterization and Differentiation of Monovarietal Grape Marc Distillates on the Basis of Varietal Aroma Compound Composition

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To investigate the varietal aroma compound composition of monovarietal grape marc distillates made from six different varieties (*Vitis vinifera* L.) in the region of Istria (Croatia), 30 samples were subjected to GC/MS and GC/FID analysis. A total of 73 compounds were identified: 45 monoterpenes, 20 sesquiterpenes, 3 diterpenes, and 5 C_{13}-norisoprenoids. The largest number and the highest concentration of monoterpenes were found in Muscat Blanc, followed by Rose Muscat of Poreč (Muškat ruža porečki) distillates, which were both characterized as highly aromatic. Lower, but still significant monoterpenol content was determined in distillates made from Istrian Malvasia (Malvazija istarska) grape marc. Chardonnay, Cabernet Sauvignon, and Teran distillates exhibited poorer monoterpene profiles, while Teran distillates contained elevated sesquiterpene concentrations. It was concluded that investigated monovarietal grape marc distillates significantly differ in varietal aroma compound composition. Stepwise linear discriminant analysis provided efficient discrimination models, and extracted various monoterpenes, sesquiterpenes and C_{13}-norisoprenoids as important differentiators of distillates according to varietal origin.

KEYWORDS: *Vitis vinifera* L.; monovarietal grape marc distillates; varietal aroma compounds; GC/MS; SLDA

INTRODUCTION

The complex aroma of fresh grape marc distillates is formed through a large array of chemical and microbiological reactions during several production steps, including grape processing, followed by ensilage, fermentation, and distillation of the marc (*1*). Past the major contribution of fermentation aroma compounds, such as higher alcohols, volatile esters, acids, and aldehydes, distillate specific aroma derives from the presence of varietal aroma compounds originating from grapes (*2–4*). If all production stages are properly conducted, varietal aroma may significantly transfer to the final distillate and reflect specific organoleptic properties of a grape variety used for production.

Generally, varietal aroma compounds present in grapes, wines, and related distillates mainly pertain to the group of terpenes. Odoriferous monoterpenols such as linalool, α-terpineol, citronellol, nerol, geraniol, and ho-trienol are usually reported as the most important because of their relatively high abundance and volatility (*5*, *6*). Responsible for flowery and fruity nuances, these compounds strongly contribute to the aroma of distillates made from aromatic, especially Muscat, varieties (*2*, *3*, *7–9*). Sesquiterpenes have also been reported as constituents of grapes (*10–12*) and grape distillates (*2*, *4*, *8*), but their contribution to the aroma of distillates has not yet been established with certitude. Another

important group of varietal aroma compounds are C_{13}-norisoprenoids, frequently reported among impact odorants in grapes and wines (*6*, *13–17*). C_{13}-Norisoprenoids such as β-damascenone, α- and β-ionone, vitispiranes, and TDN (1,1,6-trimethyl-1,2-dihydronaphthalen) have also been identified in different grape and wine spirits (*2–4*, *18*, *19*). 3-Alkyl-2-methoxypyrazines, contributors to the aroma of wines made from Sauvignon Blanc, Cabernet Sauvignon, and Semillon grapes with their earthy–peppery notes, together with benzenoid compounds, mercaptanes, and particular higher alcohols, complete the list of families of natural compounds originating from grapes often associated with the concept of varietal aroma (*6*).

Grape marc distillates are traditional spirit drinks in all European Mediterranean countries and are recognized as an important part of their national identities. Probably the most interesting and prized by the consumers are distillates produced from single varieties due to varietal aroma responsible for typicalness and recognizability of their flavor which reflects their specific origin. In contrast to wine research, relatively small number of attempts has been made to characterize monovarietal grape marc distillates on the basis of varietal aroma composition. The most studied are Italian monovarietal *grappas* and grape distillates (*1*). Di Stefano tentatively identified a large number of monoterpenes, sesquiterpenes, and norisoprenoids in Muscat *grappa* (*2*), while Da Porto et al. (*9*) investigated the influence of different production procedures on major monoterpenol composition of Muscat

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of Canelli grape distillates. Versini et al. (*8*, *20*) investigated the varietal aroma profile of Gewürztraminer *grappas* and grape distillates, while Flamini et al. (*19*) characterized *grappa* made from the Prosecco variety. Studies were also conducted in order to differentiate *grappas* made from different varieties, such as Picolit, Sauvignon, Verduzzo, Ribolla, Traminer, Refosco, Cabernet Sauvignon, and Zibibbo di Pantelleria (*4*), Gewürztraminer, Rose Muscat, Yellow Muscat, and Müller Thurgau (*8*), and Muscat, Barbera, and Nebbiolo (*21*), on the basis of varietal aroma composition. Among other Mediterranean distillates, it is worth mentioning the studies of Cortés-Diéguez et al. (*3*), who compared varietal aroma profiles of monovarietal Spanish *orujo* marc distillates made from Treixadura, Mencia, Godello, Albariño, and Catalán Roxo grape varieties, and that of Versini et al. (*22*), who characterized Catalán Roxo marc distillates on the basis of monoterpene content among other compounds.

As in the case of Italian *grappa*, Spanish *orujo*, Portuguese *bagaceira*, Greek *tsipouro*, and French *eau de vie*, the production of grape marc distillates in the Istria region of Croatia has a long tradition. It is ordinarily related to small family farms, where it is carried out using basic equipment which comprises plastic ensilage tanks and traditional copper alembics of small volume. The most important autochthonous grape varieties from which wine and marc distillates are produced are Istrian Malvasia (Malvazija istarska), being the most widespread white grape variety in Istria and the second in Croatia, Rose Muscat of Poreč (Muškat ruža porečki), commonly used for the production of rosé wines, and Teran, the most widespread red grape variety in Istria. The most significant introduced varieties for Istria are world-known Chardonnay, Muscat Blanc, and Cabernet Sauvignon.

This study was first aimed at the characterization of varietal aroma potential of monovarietal marc distillates produced from the most important grape varieties in the Istria region of Croatia. The objective was to better describe and understand the typicalness of these products in order to improve their specific quality in the future and also to differentiate them from distillates produced in other countries of Mediterranean basin. Second, the aim was to determine which varietal aroma compounds, regardless of their impact on aroma, could be used as markers of varietal origin, all in order to authenticate and protect these products which are often subject to adulteration.

MATERIALS AND METHODS

Grape Marc Samples. Grape (*Vitis vinifera* L.) varieties included in the investigation were Istrian Malvasia, Chardonnay, Muscat Blanc, Rose Muscat of Poreč, Teran, and Cabernet Sauvignon. Five marc samples of each of the six varieties, obtained in harvest 2006 in the Istria region of Croatia, were consigned voluntarily by local producers. Marcs of the same variety were obtained by different producers in order to comprise the influence of the intravarietal variability due to different growth and production conditions. Samples were obtained after winemaking procedures usual and characteristic for the variety, which included standard wine production practices: crushing, mashing and destemming of the grapes, and sulfiting. Details on further grape processing are presented in **Table 1**.

Fermentation Conditions of Marc Samples. Grape marcs were collected immediately after mashes were pressed. Seventy kg samples were transferred into appropriate 100 L plastic containers previously thoroughly cleaned. Marcs from white grape varieties and Rose Muscat of Poreč were inoculated with 20 g/100 kg of selected *Saccharomyces cerevisiae* wine yeast. Already fermented Teran and Cabernet Sauvignon marcs were also ensiled in order to keep the time interval from grape mashing to distillation the same for all samples. The surface of marc samples, which is more susceptible to oxidation, was sprayed with 5 g of the potassium meta-bisulfite and covered with plastic folium. The containers were duly sealed by pouring 25 L of water onto plastic folium in order to ensure anaerobic fermentation conditions. Fermentation was carried out over a period of 22−25 days.

Table 1. Grape Processing Parameters in the Production of Wine and Grape Marc from Six Grape Varieties (PE, Pectolytic Enzymes; MD, Maceration Duration; MT, Maceration Temperature)

grape variety	SO$_2$ (g/100 kg)	PE (g/100 kg)	yeast (g/hl)	MD (h)	MT (°C)
	grape processing parameter				
White Grape Varieties					
Istrian Malvasia 1		3			
Istrian Malvasia 2	5	3		2	18
Istrian Malvasia 3	15	2			
Istrian Malvasia 4	10	5		8	20
Istrian Malvasia 5	10				
Chardonnay 1	10	2			
Chardonnay 2	10				
Chardonnay 3	10				
Chardonnay 4					
Chardonnay 5	15				
Muscat Blanc 1	15	1			
Muscat Blanc 2	15	1			
Muscat Blanc 3	7	5		3	20
Muscat Blanc 4	10	2		72	18
Muscat Blanc 5	8	4		12	15
Red Grape Varieties					
Muscat Rose of P. 1	10	1.5		20	25
Muscat Rose of P. 2	3			24	20
Muscat Rose of P. 3	10	2		40	13
Muscat Rose of P. 4	10	2		56	27
Muscat Rose of P. 5	7	4		12	18
Teran 1[a]	10	3	19	216	28
Teran 2[a]	3		20	168	23
Teran 3[a]	4	2	20	168	26
Teran 4[a]	5	2	20	240	28
Teran 5[a]	5	2	20	240	28
Cabernet S. 1[a]	4	2	20	120	28
Cabernet S. 2[a]	10	2	17	120	26
Cabernet S. 3[a]	13	1	40	144	29
Cabernet S. 4[a]	10	3	20	216	28
Cabernet S. 5[a]	5	2	20	240	28

[a] Simultaneous maceration and fermentation.

Distillation of Fermented Marc Samples. Fermented grape marcs were distilled in a 120 L traditional copper alembic. The alembic consisted of an onion-shaped boiler in the stainless steel casing, topped by a boiler-head in the shape of a turban, and prolonged by a swan-neck tube that turned into a coil and circulated through a cooling tank. Marc was placed up on a copper grate placed on the bottom of the boiler, under which 20 L of water was previously added to prevent marc from burning. Before the beginning of distillation, the alembic was hermetically sealed in order to prevent any vapor leakage. Heating of the boiler was conducted by direct fire, with natural gas as a heating source. Distillation flow rate was kept at approximately 20 mL/min by carefully regulating the flame, and water in the cooling tank was kept between 20 and 22 °C throughout. The volume fraction of ethanol was measured in collected 100 mL fractions by an areometer. Three main distillation fractions were obtained. The fore-run part of the distillate (known as the "head" fraction), comprising approximately 5% of the volume being distilled, and the after-run part of the distillate (known as the "tail" fraction), collected after the alcohol content of a running distillate dropped under 50 vol %, were discarded. The middle-run part (the "heart" fraction) was saved as a fresh distillate. Heart fractions yielded for Istrian Malvasia from 2.4 to 3.5, for Chardonnay 1.9 to 3.3, for Muscat Blanc 1.9 to 3.6, for Rose Muscat 1.9 to 4.1, for Teran 1.8 to 2.5, and for Cabernet Sauvignon 1.7 to 2.5 L of absolute alcohol, respectively. Distillates were collected in dark bottles, stoppered, stored at 20 °C for three months, and then adjusted to 43 vol % of ethanol. Distillates were stored at 20 °C for an additional month and then analyzed.

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Chemicals. Pure standards of β-pinene, limonene, eucalyptol, linalool, α-terpineol, citronellol, nerol, geraniol, *cis*- and *trans*-furan-linalool oxides, linallyl acetate, citronellyl acetate, neryl acetate, geranyl acetate, geranic acid, nerolidol, α-ionone, β-ionone, and internal standards 3-heptanol and 3-octanol were purchased from Merck (Darmstadt, Germany) and Fluka (Buchs, Switzerland). Dichloromethane 99.8%, and sodium sulfate 99% were supplied by Kemika (Zagreb, Croatia). Pure deionized water was obtained from an Elix 3 purification system (Millipore, Bedford, MA).

Extraction and Analysis of Varietal Aroma Compounds in Grape Marc Distillates. Varietal aroma compounds were extracted from grape marc distillates by liquid–liquid extraction with dichloromethane. A 12 mL volume of a distillate sample was diluted with 150 mL of deionized water, and 75 g of ammonium sulfate were added in order to improve the extraction efficiency. A 250 μL aliquot of internal standard solution (3-octanol in ethanol, 50 mg/L) was added to control the extraction. Aroma compounds were extracted with 5 mL of dichloromethane in a 250 mL parafilm-sealed separatory funnel under vigorous agitation using an electronic shaker for a period of 90 min. Prior to organic phase separation, a closed funnel was kept in a freezer at −20 °C for 30 min. After the organic phase was separated, transferred into a screw-cup vial, and kept cold, the sample was re-extracted with two 5 mL portions of dichloromethane, following the same procedure. Dichloromethane extracts were combined, dried over anhydrous sodium sulfate, and concentrated under a gentle stream of nitrogen until the extract volume was reduced to 0.5 mL. To control injection, 10 μL of 3-heptanol ethanolic solution (1000 mg/L) was added as the second internal standard.

Identification of varietal aroma compounds was performed by GC/MS analysis using a Varian 3900 gas chromatograph coupled to a Varian Saturn 2100T ion trap mass spectrometer (Varian Inc., Harbour City, CA). The fused silica column used was a 60 m × 0.25 mm i.d. × 0.25 μm film thickness Rtx-WAX (Restek, Belafonte, PA). Two μL of the dichloromethane extract were injected in splitless mode. The GC oven parameters were as follows: initial 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 240, 80, and 120 °C, respectively. Mass spectra were acquired in the electron impact mode (70 eV) at 1 scan/s, using full scan with a mass acquisition range of 30–450 amu. Helium was used as a carrier gas with a flow rate of 1 mL/min. The identification of compounds was performed by comparing their retention times and mass spectra to those of pure standards when available and to mass spectra from NIST05 libraries. Identification by comparison with mass spectra was considered satisfactory only for compounds with obtained spectra reverse match numbers higher than 800. Additional identification was achieved by comparing linear retention indices, calculated for identified compounds (relative to *n*-alkanes), to those from literature.

Quantitative determination of varietal aroma compounds was performed using a Varian 3350 gas chromatograph equipped with a flame-ionization detector (FID). The column and GC oven parameters were the same as previously described. Injector and detector temperatures were 240 and 245 °C, respectively. Two μL of the dichloromethane extract were injected in splitless mode, and the carrier gas was helium with a flow rate of 1 mL/min. Detector gases were hydrogen (with a flow rate of 30 mL/min), air (300 mL/min), and helium as makeup gas (30 mL/min). Calibration curves (relative peak area versus concentration ratio of aroma compound/internal standard 3-heptanol) were constructed when standards were available, and quantification was performed by the internal standard method using Varian Star 4.51 software. For other compounds, semiquantitative analysis was carried out, and concentrations were calculated as mg/L of internal standard 3-octanol, assuming a response factor equal to one.

The efficacy of the extraction procedure was checked by performing five extractions of each of five model standard solutions containing different concentrations of chemical standards. Concentrations of standards were chosen in order to encompass the concentration ranges found in literature. Model solutions contained 43% vol of ethanol, 360 mg/L of acetic acid, 225 mg/L of acetaldehyde, 1000 mg/L of ethyl acetate, 1000 mg/L of methanol, 275 mg/L of 1-propanol, 350 mg/L of isobutanol, and 1250 mg/L of isoamyl alcohols in order to more accurately simulate a real distillate sample matrix. Method limits of detection (LOD) were estimated as three, while limits of quantification (LOQ) were estimated as 10 times the baseline noise recorded on GC/FID, respectively. Because detection and quantification limits of individual compounds changed along with the FID

Table 2. Concentration Ranges, Correlation Coefficients of Calibration Curves, Extraction Recoveries, and Relative Standard Deviations of Recovery, Limits of Detection (LOD), and Quantification (LOQ) for the Extraction of Standards of Volatile Compounds from Model Solutions (*n* = 25)

varietal aroma compound	range (mg/L)	r^2	recovery (%) (RSD)	LOD (mg/L)	LOQ (mg/L)
Monoterpenes					
β-pinene	0.1–12	0.9993	90 (4)	0.012	0.040
limonene	0.1–12	0.9989	95 (4)	0.017	0.057
eucalyptol	0.1–12	0.9988	92 (7)	0.012	0.040
trans-linalool-furan-oxide	0.1–12	0.9984	98 (6)	0.008	0.027
cis-linalool-furan-oxide	0.1–12	0.9980	92 (4)	0.013	0.043
linalool	1–120	0.9998	99 (8)	0.015	0.050
citronellyl acetate	0.1–12	0.9981	93 (3)	0.011	0.036
α-terpineol	1–120	0.9984	95 (3)	0.016	0.053
neryl acetate	0.1–12	0.9979	95 (3)	0.015	0.050
geranyl acetate	0.1–12	0.9984	91 (8)	0.012	0.040
citronellol	1–120	0.9991	100 (2)	0.012	0.040
nerol	1–120	0.9991	101 (3)	0.014	0.047
geraniol	1–120	0.9986	98 (2)	0.012	0.040
geranic acid	0.1–12	0.9976	90 (8)	0.019	0.063
Sesquiterpenes					
trans-nerolidol	0.1–12	0.9988	91 (7)	0.019	0.063
C₁₃-Norisoprenoids					
β-damascenone	0.1–12	0.9977	92 (5)	0.020	0.067
α-ionone	0.1–12	0.9987	93 (5)	0.012	0.040
β-ionone	0.1–12	0.9984	95 (6)	0.013	0.043
Internal Standard					
3-octanol			99 (3)		

sensitivity, average limits registered during method validation were reported. Validation parameters of the method are reported in **Table 2**.

Statistical Analyses. All analyses were performed in duplicate, and average values were used in further data elaboration. Concentration means and standard deviations were calculated from five replicates, i.e., five samples for each investigated variety. One-way analysis of variance (ANOVA) was carried out using Microsoft Excel (Microsoft, Seattle, WA), and least significant difference test was used to compare the means at the level of significance of $p < 0.05$. To differentiate grape marc distillates according to varietal origin, stepwise linear discriminant analysis (SLDA) was carried out using Statistica 5.0 software (StatSoft Inc., Tulsa, OK).

RESULTS AND DISCUSSION

Varietal aroma compounds identified and quantified in mono-varietal grape marc distillates are listed in **Tables 3** and **4**. A total of 73 compounds were identified: 45 monoterpenes, 20 sesquiterpenes, 3 diterpenes, and 5 C₁₃-norisoprenoids. In preliminary investigation, an attempt was made to identify methoxypyrazines, especially in Cabernet Sauvignon distillates, because grapes and wines from this variety are known to contain relevant amounts of these compounds (*6*, *23*). However, no methoxypyrazines were identified, so it was assumed that their concentrations were below detection limits of the method.

The concentrations of compounds identified in this study were approximately 2 orders of magnitude higher than those reported for different types of wine (*6*), including compounds that are not usually detected in wine. There are many known causes for such phenomena. Although a portion of varietal aroma transfers to a distillate in volatile form which was present in grapes, during production of grape marc distillates, such compounds undergo many transformations. Because a significant part of terpenes and C₁₃-norisoprenoids is present in marc in glycosidically bound form, the most important is their enzymatic and chemical hydrolysis

Table 3. Concentration Means ± Standard Deviations (mg/L) of Monoterpenes in Monovarietal Grape Marc Distillates Produced in the Istria Region of Croatia[a]

varietal aroma compound	RI	RM	FM	Istrian Malvasia	Chardonnay	Muscat Blanc	Rose Muscat of Poreč	Teran	Cabernet Sauvignon
						Monoterpenes			
lilac alcohol C[b]	1096	810	752			0.05 a ± 0.02			
epoxylinalool[c]	1111	823	815			2.26 a ± 0.24	1.81 b ± 0.78		
δ-3-carene[c]	1147	889	889			0.06 b ± 0.02	0.13 a ± 0.03		
β-myrcene[c]	1162	884	798			0.29 a ± 0.08	0.22 a ± 0.10		
limonene[d]	1196	892	872	0.03 c ± 0.01		0.24 a ± 0.08	0.10 b ± 0.05		
eucalyptol[d]	1214	887	885			detected	detected		
trans-dihydrocarvone[b]	1224	801	789			0.83 a ± 0.22	0.00 b ± 0.00		
trans-β-ocimene[c]	1253	874	803			0.68 a ± 0.23	0.56 a ± 0.15		
α-terpinolene[c]	1281	904	876	0.08 c ± 0.03		0.74 a ± 0.23	0.47 b ± 0.04	0.02 c ± 0.02	
trans-2-pinanol[b]	1327	862	777	0.11 c ± 0.07		1.18 a ± 0.33	0.84 b ± 0.48		
trans-rose oxide[c]	1367	885	835			0.04 a ± 0.01	0.02 b ± 0.01		
trans-linalool-furan-oxide[d]	1436	881	737	0.28 c ± 0.09		2.73 a ± 0.79	1.30 b ± 0.35		
terpene (unidentified)[b]	1446					0.33 a ± 0.11	0.49 a ± 0.35		
cis-linalool-furan-oxide[d]	1464	903	808	0.14 c ± 0.05	0.13 c ± 0.08	1.97 a ± 1.02	0.72 b ± 0.29	0.04 c ± 0.01	0.03 c ± 0.02
nerol oxide[c]	1467	857	819			0.89 a ± 0.51			
terpene (unidentified)[b]	1476			0.03 c ± 0.01		0.21 a ± 0.04	0.12 b ± 0.02		
6-isopropylidene-1-methyl-bicyclo(3.1.0)hexane[b]	1482	836	785			0.21 a ± 0.02	0.13 b ± 0.12		
α-terpinene[b]	1506	855	855	0.04 b ± 0.03		0.13 a ± 0.07	0.17 a ± 0.14		
lilac aldehyde B[b]	1531	802	722			0.03 a ± 0.01			
linalool[d]	1542	866	838	3.08 bc ± 1.14	0.40 c ± 0.20	34.94 a ± 17.79	12.12 b ± 4.23	0.14 c ± 0.07	0.12 c ± 0.04
4-terpineol[c]	1593	925	879			0.40 a ± 0.09	0.29 b ± 0.08		
ho-trienol[c]	1601	818	818	0.09 b ± 0.19		4.29 a ± 1.31	0.48 b ± 0.24		
trans-β-terpineol[c]	1620	890	812			0.07 a ± 0.02	0.02 b ± 0.01		
citronellyl acetate[d]	1645	882	832			0.29 a ± 0.05	0.13 b ± 0.02		
cis-sabinene hydrate acetate[b]	1667	870	789			0.24 a ± 0.13			
trans-shisool[b]	1673	820	782			0.33 a ± 0.12	0.06 b ± 0.04		
terpene (unidentified)[b]	1673					0.20 a ± 0.09			
α-terpineol[d]	1684	886	862	0.59 c ± 0.09	0.32 cd ± 0.12	6.34 a ± 0.96	2.98 b ± 0.48		
terpinenyl acetate[c]	1688	862	826			0.10 a ± 0.02	0.03 b ± 0.04		
neryl acetate[e]	1721	845	785			0.12 a ± 0.03			
trans-linalool-pyran-oxide[c]	1726	834	815			1.54 a ± 0.27	0.01 b ± 0.02		
ethyl geranate[b]	1737	814	794			0.43 a ± 0.11	0.03 b ± 0.06		
geranyl acetate[e]	1749	801	779			0.31 a ± 0.07	0.18 b ± 0.06		
cis-linalool-pyran-oxide[c]	1752	822	772			0.62 a ± 0.40			
citronellol[d]	1758	842	802	0.39 c ± 0.12	0.09 c ± 0.03	4.56 a ± 1.52	3.12 b ± 1.55	0.20 c ± 0.05	0.17 c ± 0.03
nerol[d]	1791	908	815	0.13 c ± 0.08		3.98 a ± 1.64	1.45 b ± 0.41		
lilac alcohol D[b]	1816	803	711			0.03 a ± 0.01			
geraniol[d]	1838	876	820	0.55 c ± 0.26	0.20 c ± 0.07	6.45 a ± 1.74	2.88 b ± 1.10	0.07 c ± 0.03	0.10 c ± 0.03
geranyl acetone[c]	1845	916	849	0.07 b ± 0.07		0.26 a ± 0.17	0.18 a ± 0.03		
cis-p-mentha-2,8-dien-1-ol[b]	1871	801	777			0.04 a ± 0.02			
p-menth-1-en-9-ol[c]	1920	844	828			0.05 a ± 0.01			
geranyl isovalerate[b]	1943	801	750			0.08 a ± 0.02	0.03 b ± 0.02		
3,7-dimethyl-1,7-octadien-3,7-diol[c]	1962	804	804			0.07 a ± 0.02			
p-mentha-1(7),8(10)-dien-9-ol[b]	1981	833	824	0.03 b ± 0.01		0.09 a ± 0.01	0.01 c ± 0.01		
geranic acid[d]	2319	847	833	0.04 c ± 0.05		2.03 a ± 0.51	0.33 b ± 0.04		
total monoterpenes				5.62 c ± 1.21	1.13 c ± 0.26	80.81 a ± 18.17	31.40 b ± 4.82	0.47 c ± 0.09	0.42 c ± 0.06

[a] Lower case letters on line indicate significant differences between means within rows at the level of significance of *p* < 0.05 determined by one-way analysis of variance (ANOVA) and Least Significant Difference (LSD) comparison test. RI, calculated linear retention indices; RM, mass spectra reverse match number; FM, mass spectra forward match number. [b] Mass spectra consistent with those from the NIST05 electronic library (tentative identification). [c] Mass spectra consistent with those from the NIST05 electronic library, and retention indices (RI) consistent with those found in literature. [d] Retention time and mass spectra consistent with those of the pure standards, mass spectra consistent with those from the NIST05 electronic library, and retention indices (RI) consistent with those found in literature. [e] Retention time and mass spectra consistent with those of the pure standards, and mass spectra consistent with those from the NIST05 electronic library.

which occurs during ensilage, fermentation, and distillation (*4*). Relatively long ensilage and fermentation imply a lengthy maceration of solid parts of the grapes during which aroma compounds are extracted from grape berry skins, where the highest concentrations are contained (*24*). Once glycosidically bound compounds reach liquid medium, they are susceptible to hydrolysis by enzymes and are liberated in volatile form (*3*, *4*). In many grape marc samples investigated in this work, a portion of

pectolytic enzymes was added, which are known to increase the rate of the liberation of some monoterpenes (*9*).

During ensilage and fermentation, varietal aroma compounds can be metabolized by yeasts or bacteria and transformed (*25*–*27*). It is known, for example, that during fermentation notable amounts of citronellol, linalool, and α-terpineol are produced from geraniol (*4*, *27*). The content of geraniol was shown to additionally decrease due to its participation in the

Table 4. Concentration Means ± Standard Deviations (mg/L) of Sesquiterpenes, Diterpenes and C_{13}-Norisoprenoids in Monovarietal Grape Marc Distillates Produced in the Istria Region of Croatia[a]

varietal aroma compound	RI	RM	FM	Istrian Malvasia	Chardonnay	Muscat Blanc	Rose Muscat of Poreč	Teran	Cabernet Sauvignon
Sesquiterpenes									
α-ylangene[c]	1479	906	906	0.47 ab ± 0.46	0.07 bc ± 0.04	0.06 bc ± 0.03	0.34 bc ± 0.36	0.82 a ± 0.56	0.04 c ± 0.03
α-burbonene[c]	1513	917	875	0.08 b ± 0.11	0.09 b ± 0.10		0.28 ab ± 0.30	0.38 a ± 0.42	
β-ylangene[c]	1568	829	810	0.04 a ± 0.03	0.02 ab ± 0.02		0.03 ab ± 0.03	0.05 a ± 0.03	0.00 b ± 0.00
trans-α-bergamotene[c]	1582	912	821		0.08 a ± 0.06				
β-copaene[c]	1585	809	757	0.05 ab ±0.04	0.03 ab ±0.02	0.01 b ± 0.01	0.09 a ± 0.08	0.09 a ± 0.07	
isoledene[b]	1616	867	867	0.14 b ± 0.13	0.02 b ± 0.02	0.04 b ± 0.02	0.12 b ± 0.10	0.43 a ± 0.35	0.03 b ± 0.03
germacrene D[c]	1702	820	788	0.04 b ± 0.06	0.05 b ± 0.03	0.18 b ± 0.06	0.34 ab ± 0.28	0.83 a ± 0.94	0.05 b ± 0.03
β-selinene[c]	1707	872	795	0.11 ab ± 0.09	0.03 b ± 0.04	0.03 b ± 0.02	0.07 ab ± 0.06	0.13 a ± 0.10	0.05 b ± 0.03
α-muurolene[c]	1715	877	877	0.12 ab ± 0.08	0.04 b ± 0.03	0.10 ab ± 0.06	0.17 a ± 0.13	0.13 ab ± 0.13	0.03 b ± 0.01
α-farnesene[c]	1743	857	824	0.04 b ± 0.06		0.15 a ± 0.03		0.05 b ± 0.04	0.03 bc ±0.01
γ-cadinene[c]	1752	897	841	0.38 b ± 0.29	0.20 b ± 0.16	0.28 b ± 0.08	0.54 b ± 0.58	2.32 a ± 1.83	0.06 b ± 0.03
α-gurjunene[c]	1770	854	762	0.04 bc ± 0.05	0.02 bc ± 0.01	0.03 bc ± 0.02	0.07 ab ± 0.06	0.13 a ± 0.09	
nerolidyl acetate[b]	1818	801	736					0.04 a ± 0.02	0.03 a ± 0.01
trans-calamenene[c]	1821	855	855	0.02 b ± 0.03	0.01 b ± 0.00	0.00 b ± 0.01	0.04 b ± 0.02	0.13 a ± 0.11	0.02 b ± 0.01
calacorene[c]	1903	885	882	0.15 ab ± 0.16	0.07 b ± 0.02	0.06 b ± 0.02	0.16 ab ± 0.15	0.25 a ± 0.25	0.04 b ± 0.03
trans-nerolidol[d]	2031	901	868	0.07 c ± 0.03	0.09 c ± 0.04	0.10 c ± 0.03	0.10 c ± 0.06	0.58 a ± 0.22	0.42 b ± 0.08
τ-muurolol[c]	2182	864	854	0.05 ± 0.03	0.05 ± 0.08	0.02 ± 0.01	0.05 ± 0.03	0.07 ± 0.07	
aristolene[b]	2194	817	740	0.10 ab ± 0.10	0.14 a ± 0.13	0.02 b ± 0.01	0.05 ab ± 0.02	0.07 ab ± 0.04	0.01 b ± 0.01
α-cadinol[c]	2214	874	869	0.05 ± 0.04	0.03 ± 0.02	0.01 ± 0.01	0.11 ± 0.16	0.07 ± 0.12	0.00 ± 0.00
α-farnesol[c]	2341	888	877	0.21 c ± 0.07	0.21 c ± 0.08	0.34 c ± 0.06	0.23 c ± 0.05	1.05 a ± 0.43	0.75 b ± 0.18
total sesquiterpenes				2.16 b ± 0.63	1.25 b ± 0.28	1.44 b ± 0.15	2.80 b ± 0.86	7.64 a ± 2.28	1.57 b ± 0.21
Diterpenes									
isophytol[b]	2284	828	828	0.04 b ± 0.03	0.05 b ± 0.03	0.03 b ± 0.04	0.04 b ± 0.02	0.13 a ± 0.08	0.12 a ± 0.03
manoyloxide[c]	2344	868	858	0.61 ab ± 0.31	0.57 ab ± 0.41	0.25 b ± 0.13	1.15 a ± 0.83	0.81 ab ± 0.51	0.42 b ± 0.21
phytol[c]	2603	866	859	0.68 a ± 0.04	0.50 ab ± 0.09	0.40 b ± 0.10	0.42 b ± 0.04	0.65 a ± 0.17	0.63 a ± 0.18
total diterpenes				1.33 a ± 0.31	1.12 ab ± 0.42	0.68 b ± 0.17	1.61 a ± 0.83	1.59 a ± 0.54	1.17 ab ± 0.28
C_{13}-Norisoprenoids									
vitispirane A[e]	1521			0.20 b ± 0.08	0.49 a ± 0.29	0.22 b ± 0.06	0.50 a ± 0.40	0.12 b ± 0.03	0.09 b ± 0.05
vitispirane B[e]	1523			0.19 bc ± 0.08	0.64 a ± 0.42	0.23 bc ± 0.08	0.44 ab ± 0.36	0.11 c ± 0.03	0.08 c ± 0.05
TDN[c]	1731	900	821	0.05 ± 0.02	0.06 ± 0.03	0.05 ± 0.01	0.05 ± 0.05	0.04 ± 0.01	0.03 ± 0.01
β-damascenone[d]	1809	829	785	0.08 bc ± 0.02	0.08 bc ± 0.02	0.12 a ± 0.03	0.07 bc ± 0.03	0.09 ab ± 0.03	0.05 c ± 0.02
β-ionone[d]	1923	891	841	0.07 b ± 0.03	0.05 bc ± 0.02	0.07 b ± 0.02	0.02 c ± 0.04	0.11 a ± 0.02	0.11 a ± 0.01
total C_{13}-norisoprenoids				0.59 bc ± 0.12	1.32 a ± 0.51	0.69 bc ± 0.11	1.07 ab ± 0.54	0.46 bc ± 0.06	0.36 c ± 0.07

[a] Lower case letters on line indicate significant differences between means within rows at the level of significance of $p < 0.05$ determined by one-way analysis of variance (ANOVA) and least significant difference (LSD) comparison test. RI, calculated linear retention indices; RM, mass spectra reverse match number; FM, mass spectra forward match number. [b] Mass spectra consistent with those from the NIST05 electronic library (tentative identification). [c] Mass spectra consistent with those from the NIST05 electronic library, and retention indices (RI) consistent with those found in literature. [d] Retention time and mass spectra consistent with those of the pure standards, mass spectra consistent with those from the NIST05 electronic library, and retention indices (RI) consistent with those found in literature. [e] Mass spectra consistent with those found in literature, and retention indices (RI) consistent with those found in literature.

sterol biosynthetic pathway (27). The case of eucalyptol (1,8-cineole), a monoterpenol found in very high concentrations in monovarietal *orujo* distillates (3), is also interesting. This compound was identified but not quantified in this work due to strong peak overlapping with isoamyl alcohol (**Table 3**). In a recent investigation, the authors suggested that eucalyptol derives from chemical transformations of limonene and α-terpineol (28). Esterification reactions between terpenols and acetic acid (2) and between alcohols and geranic acid (8) also take place. During fermentation, some odorless C_{13}-norisoprenoid aglycons released after enzymatic hydrolysis of glycoconjugated precursors produced by degradation of carotenoids can be furtherly transformed in odoriferous norisoprenoids by chemical hydrolysis in acidic medium (4, 6, 17).

Distillation high temperatures, especially when supported by acidic conditions, are known to catalyze different intramolecular conversions, and induce the hydrolysis of glycosides and polyols (2, 4, 9, 29, 30). Myrcenol and ocimenols are known to

originate from 3,7-dimethyl-1-octen-3,7-diol and 3,7-dimethyl-1,7-octadien-3,6-diol (2), while it was presumed that furanic oxides of linalool derive from 2,6-dimethyl-7-octen-2,3,6-triol (31). The thermal dehydration of 2,6-dimethyl-3,7-octadiene-1,6-diol leads to the formation of ho-trienol (32). Williams et al. (31) found in model experiments that 3,7-dimethyl-1,5-octadiene-3,7-diol rearranged under acidic conditions to give ho-trienol and nerol oxide. Factors that affect the rate of hydrolysis of glycosides and transformations of aglycons during distillation are distillation flow rate and pH of the marc. If distillation is slow, as in the case of discontinuous distillation in this investigation, there is more time for mentioned reactions to take place. On the other hand, elevated pH of the marc that was not acidified, as in this work, limits chemically induced hydrolysis. These conditions are especially critical for the release of volatile C_{13}-norisoprenoids, which are present in grapes almost exclusively in the form of glycosides (4). During distillation, sesquiterpenes are released from wax layers of grape berry skins (8), which is not achievable during

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winemaking and results in negligible concentrations of sesquiterpenes in wine (*11*). By distillation, volatile compounds from grapes and marc are concentrated. Finally, the composition of distillates is significantly affected by the behavior of volatiles during distillation, determined by their boiling points and solubility in water and ethanol present in liquid and vapor phase.

In light of the above discussion, it was assumed that not only the absolute but also the relative concentrations of the identified compounds in investigated distillates significantly changed in relation to raw material, that is the grapes.

Relatively high standard deviations of concentration means observed for the majority of compounds reported in **Tables 3** and **4** imply a certain intravarietal variability in composition, which is due to many inevitable sources of variation. Grape quality, as a result of *terroir* and vine growing conditions, together with differences in the application of various winemaking techniques, such as maceration, use of pectolytic enzymes, and pressing, certainly affected final composition of the marcs (*5, 6, 33*).

Beyond the intravarietal differences, the results of one-way ANOVA analysis shown in **Tables 3** and **4** suggest that marc distillates produced from different grape varieties significantly differ in varietal aroma compound composition.

Monoterpenes. Results presented in **Table 3** sorted out Muscat Blanc grape marc distillates as those with the most complex monoterpene composition. In mentioned distillates, the largest number of monoterpenes was identified, present in the highest concentrations in relation to distillates made from other varieties, in most cases with statistically significant difference. Distillates made from Rose Muscat of Poreč grape variety were also characterized by very high monoterpene content, although notably less abundant than in the case of Muscat Blanc. Qualitatively, monoterpene composition of two Muscat distillates coincided in a significant measure with that determined by Di Stefano for Muscat *grappa* (*2*).

Monoterpenes found in the highest concentrations were monoterpenols linalool, α-terpineol, citronellol, nerol, geraniol, and ho-trienol, which are usually reported as the major varietal aroma compounds in distillates made from Muscat varieties (*2, 7−9*) and are responsible for distinguishable flowery−fruity flavor also known as Muscat aroma (*34*). Particular odors of linalool, nerol, and geraniol are usually described as reminiscent of roses, while odor of α-terpineol is compared with the scents of lily and camphor (*6*). Citronellol is considered to be responsible for citrus notes, and ho-trienol odor resembles honey and linden (*6, 21*).

Monoterpenol concentrations found in Muscat distillates in this investigation are comparable to high amounts determined in aromatic grape and wine distillates in previous works (*3, 7−9, 19, 35*), indicating that their influence on the aroma is dominant. In comparison to Mediterranean grape marc distillates, investigated Muscat distillates emerged among the most aromatic due to very high monoterpenol content. For example, samples of monovarietal *orujo* contained linalool concentrations up to 7.87 mg/L (*3*), while concentrations found in *grappas* made from Muscat of Canelli (*9*) and Prosecco (*19*) grape varieties were 31.98 and 10.506 mg/L of absolute alcohol, respectively.

Relatively high concentrations of *cis* and *trans* isomers of both furanoid and pyranoid linalool oxides were found in Muscat Blanc, while only trace amounts of pyranoid oxides were found in Rose Muscat distillates. Several other components were found unique for Muscat Blanc. Obviously, composition of two Muscat distillates rich in monoterpenes markedly differed, meaning that subtle differences in the aroma of these products may be expected, although still being of typical Muscat character. The influence of linalool oxides on aroma is generally considered marginal due to their high perception thresholds in wine (*5, 6*). However, their

contribution in spirit drinks is still unknown and requires a further investigation.

Many minor monoterpenes were found in concentrations below 1 mg/L in the majority of samples. Although the organoleptic role of different monoterpene hydrocarbons such as limonene, δ-3-carene, *trans-β*-ocimene, α-terpinolene, and α-terpinene and some minor monoterpenols has not yet been clearly established, it is possible to presume that many of these compounds may contribute to the complexity of Muscat distillates aroma. For example, *β*-pinene and eucalyptol are both carriers of mint and eucalyptus nuances, δ-3-carene, limonene, and *trans-β*-ocimene may contribute with citrus notes, while α-terpinolene, *trans*-2-pinanol, and α-terpinene have similar odors described as woody, piney, and terpenic. Moreover, the fact that by excluding the major monoterpenols and linalool oxides, the average total concentration of the remaining monoterpenes in Muscat Blanc distillates surpassed 14 mg/L (**Table 3**) should not be neglected.

A number of monoterpene derivatives was identified in Muscat marc distillates, comprising three esters of monoterpene alcohols and acetic acid, and ethyl geranate, an ester of ethanol and geranic acid. The presence of these compounds, although formed during alcoholic fermentation, may be considered a varietal characteristic because it is directly related to the occurrence and abundance of their precursors, i.e. monoterpene alcohols and acids.

The number and concentrations of monoterpenes determined in Istrian Malvasia marc distillates were notably lower than those in Muscat distillates but still high enough to assume that their impact on the aroma could be significant. This is corroborated by the fact that determined concentrations were in the same order of magnitude as those determined in particular samples of *Pisco*, Chilean wine distillate produced from aromatic grape varieties (*36*). Significant monoterpene content in Malvasia distillates could turn out to be the important feature which distinguishes them from neutral, nonaromatic distillates. As in the case of Muscat distillates, the most abundant were the major monoterpenols, which is in accordance with the earlier findings where wines from Istrian Malvasia were characterized by a notable monoterpenol potential, with a particular contribution of linalool and geraniol (*37*). Relatively high monoterpene content in Malvasia distillates could be partially due to the excessive use of pectolytic enzymes during crushing and mashing of the grapes, which continued their activity in ensilaged marc (*9*). Another reason could be a higher degree of pressing. Both of these marc-exhausting procedures are often required during production of wine from this variety because of its thick grape berry skin and dense pulp, both rich in pectin matter (*38, 39*).

The concentrations of a few identified monoterpenes in distillates made from Chardonnay, Teran, and Cabernet Sauvignon grape marcs were quite low and were comparable to low concentrations previously determined in grape spirits made from nonaromatic varieties (*3, 35*). It was presumed that their influence on the aroma of these distillates is negligible due to strong suppression by fermentation aroma compounds. Although without statistical significance, concentrations of individual monoterpenes except citronellol, as well as total concentration, were found to be higher in Chardonnay distillates. This result is in agreement with previous findings where varietal aroma of Chardonnay was found to be more pronounced than in the case of Cabernet Sauvignon *grappa*, with linalool as the most abundant, followed by α-terpineol (*4*). It is worth mentioning that a significant portion of already limited varietal aroma potential of red grape Teran and Cabernet Sauvignon marcs was probably lost in the production of wine due to extraction into must during relatively long simultaneous maceration and fermentation.

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Sesquiterpenes. Although their presence was confirmed several times (*2*, *4*, *8*), sesquiterpenes were rarely reported as the indicators of varietal origin of grape distillates. Versini et al. (*8*) showed that the amounts of several sesquiterpenes, especially farnesol and α-farnesene isomers, could be useful variables in differentiation of Gewürztraminer from some other monovarietal *grappas*. Recent study on volatile components in monovarietal grape distillates also implicated that sesquiterpene composition could be a varietal characteristic (*12*).

Results of this investigation showed that distillates made from Teran marc contained the highest concentrations of the majority of sesquiterpenes, in most cases with statistically significant difference (**Table 4**). Ruberto et al. (*12*) established that grape stems contain a larger number of these compounds in relation to skins, pulp, and seeds, so it was presumed that sesquiterpene composition of a produced distillate may be considerably influenced by the amount of residual stems in marc. Content of stems mostly depends on how destemming was performed but could also depend on the constitution of the stem, characteristic for a particular variety. Since it is known that Teran grape stem is firm and partially wooden and therefore easily removable in destemming procedure (*39*), a significant amount of stems is not expected to remain in the corresponding marc. In fact, no excessive stem content was observed in Teran marcs during the investigation. Another fact to consider was that sesquiterpenes are prevalently contained in the wax layers of grape berry skins (*8*), so their levels in marc and distillate can be increased by applying higher pressurage degree during pressing of grape pomace. In this work, no significant correlation between the concentrations of sesquiterpenes and the indicators of pressing degree such as methanol and 1-hexanol (*8*) were observed (data not shown). In light of the above discussion, it was concluded that higher sesquiterpene content in Teran distillates could be a varietal characteristic.

Except in Cabernet Sauvignon distillates, sesquiterpene found in the highest average concentration in relation to others was γ-cadinene. The highest concentrations of *trans*-nerolidol and α-farnesol were found in distillates made from nonaromatic red grape varieties Teran and Cabernet Sauvignon. Consequently, an ester of nerolidol and acetic acid formed during fermentation, nerolidyl-acetate, was identified only in these samples. Some other sesquiterpenes were found in the highest levels in distillates made from other varieties such as aristolene in Chardonnay and α-cadinene in Rose Muscat distillates. *trans*-α-Bergamotene was identified only in Chardonnay. It is worth mentioning that this compound was previously identified in Muscat *grappa* (*2*).

Because of their weaker polarity and lower solubility in water, sesquiterpenes may act as causative agents of turbidity and flocculation, especially at lower temperatures and lower ethanol content, and therefore may be important factors of distillate instability. It is known that their content can be significantly reduced by chill filtration process, which is sometimes necessary in order to eliminate unwanted opalescence and turbidity (*40*), so regardless of obviously the highest sesquiterpene potential of Teran grapes, the results obtained should be taken with caution because they refer to fresh, raw distillates.

Sesquiterpene odors are generally described as woody, spicy, sweet, floral, clove, oily, musty, and fresh (*11*), but their contribution to the aroma of grapes, wine, and related distillates has not yet been established with certitude. In fact, these compounds were rarely reported as important odorants, an exception being the work of Goiris et al. (*41*), who attributed them the contribution to the spicy hop character of beer. Considering relatively low concentrations of sesquiterpenes found in distillates analyzed in this investigation, together with lower volatility in relation to monoterpenes, it was generally presumed that their

influence on the aroma of the majority of distillates is marginal. However, exceptionally high amounts of total sesquiterpenes, such as 15.61 mg/L found in particular Teran distillate (data not shown), imply that a certain influence is possible.

Diterpenes. Diterpenes are rather less important compounds for the aroma of distillates because of their low volatility. Judging on low concentrations presented in **Table 4**, it was presumed that their impact on the aroma of investigated distillates is negligible. It is worth mentioning higher isophytol concentrations found in distillates made from nonaromatic red grape varieties Teran and Cabernet Sauvignon.

C_{13}-Norisoprenoids. Concentrations of C_{13}-norisoprenoids determined in monovarietal grape marc distillates are presented in **Table 4**.

Higher concentrations of vitispiranes A and B were found in distillates made from Chardonnay and Rose Muscat of Poreč marcs, so it was assumed that their recognizable camphor-like odor is more likely to be observed in these products.

Even though β-damascenone is usually mentioned in the context of red wine (*6*, *14*−*17*) and Chardonnay aroma (*13*), the highest average concentration was determined in Muscat Blanc marc distillates. This compound is usually reported to be responsible for odors in wine reminiscent of honey, dried plum, and stewed apple (*15*, *17*). It is possible that β-damascenone was partially responsible for honey notes previously observed in distillates made from Muscat varieties, which were not, according to the authors, explainable only by the presence and the amounts of ho-trienol, a monoterpenol with similar odor (*21*).

The highest average concentrations of β-ionone were found in Teran and Cabernet Sauvignon distillates. This result is in agreement with findings of several authors who showed that this compound is important for red wines, among others Cabernet Sauvignon, where it is considered to be one of the key odorants contributing with the scent reminiscent of violets (*6*, *14*, *15*).

Concentrations of C_{13}-norisoprenoids determined in this work were relatively low when compared to distillates made from other particular varieties. For example, Cortés-Diéguez et al. (*3*) determined the presence of α-ionone and teaspirane in concentrations up to 1.32 and 0.62 mg/L, respectively, in different samples of monovarietal *orujo*, while Flamini et al. (*19*) found relatively high vitispirane concentration of 1.9 mg/L in *grappa* made from Prosecco. Moreover, α-ionone, actinidols and Riesling acetal that were previously identified in different monovarietal marc distillates (*3*, *4*) were not found. Considering extremely limited knowledge about the impact of norisoprenoids on distillate aroma, it was only possible to assume that relatively low amounts found in this work, being suppressed by monoterpenes and fermentation aroma compounds, contribute to its complexity.

Differentiation of Monovarietal Grape Marc Distillates According to Variety by Stepwise Linear Discriminant Analysis. As it was shown in previous paragraphs, investigated grape marc distillates produced from different grape varieties generally differ in varietal aroma compound composition. It is obvious that highly aromatic Muscat Blanc and Rose Muscat of Poreč, together with Istrian Malvasia distillates, can be easily differentiated from others on the basis of the number and the concentrations of identified monoterpenes (**Table 3**). However, the differentiation between mentioned varieties is not completely clear on the basis of ANOVA results because the concentration ranges of many compounds overlap, especially in the case of two Muscat varieties. The same applies for the group of distillates with poorer monoterpene composition, i.e. Chardonnay, Teran, and Cabernet Sauvignon. For a more reliable statistical differentiation of distillates according to varietal origin, forward stepwise discriminant analysis (SLDA) was applied separately on two sets of data.
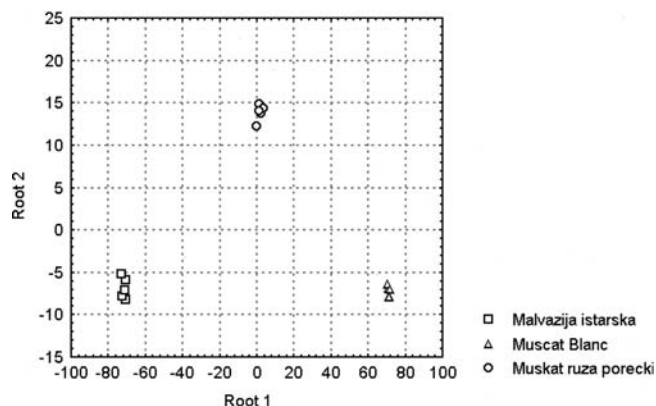
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Figure 1. Projection of Istrian Malvasia, Muscat Blanc, and Rose Muscat of Poreč grape marc distillate samples, classified according to varietal origin, along the directions of two discriminant functions.

Table 5. Standardized Coefficients of the Variables Contributed to the Differentiation of Istrian Malvasia, Muscat Blanc and Rose Muscat of Poreč Grape Marc Distillates Obtained by SLDA

variable	root 1	root 2
α-terpineol	14.7791	3.1628
geranic acid	8.2990	−0.1622
p-mentha-1(7),8(10)-dien-9-ol	0.3532	−2.8014
β-damascenone	−2.7120	−2.8174
γ-cadinene	−0.7763	3.2362
ho-trienol	9.8005	−2.4460
germacrene D	5.2178	2.5802
vitispirane B	2.8691	1.0049

The first set comprised more aromatic varieties and the second less aromatic ones. Variables which were included in building of differentiation models were those identified in at least one sample from each of the investigated varieties.

In the first case, SLDA classified 100% of all Istrian Malvasia, Muscat Blanc, and Rose Muscat of Poreč distillates according to varietal origin correctly, which is shown in **Figure 1**. Eight aroma compounds were selected according to Wilks' Lambda criterion. The first compound that entered the classification model was α-terpineol, which emerged as the most important, because 100% of all samples were classified correctly after this step. Other variables that were included in the model are listed in **Table 5**, together with the corresponding standardized coefficients. It is obvious that distillates were differentiated along the direction of the first discriminant function mainly by the concentrations of α-terpineol, geranic acid, ho-trienol, and germacrene D, while the variables contributing most to the differentiation of Rose Muscat from other distillates along the second function were α-terpineol, γ-cadinene, germacrene D (positive scores), *p*-mentha-1(7),8(10)-dien-9-ol, β-damascenone, and ho-trienol (negative scores). The prediction capacity of the SLDA model was evaluated by "leave-one-out" cross-validation where each distillate sample was removed from the model and classified by the functions derived from all cases other than that case. **Table 6** summarizes the results of the classification matrix of the original SLDA model and cross-validation procedure, showing average percentages of correct classification and correct prediction of 100.0%.

By applying SLDA to the second data set, a 100% correct classification of Chardonnay, Teran, and Cabernet Sauvignon distillates was also achieved (**Figure 2**). Eight variables were selected according to Wilks' Lambda criterion (**Table 7**). β-Ionone entered model the first and classified correctly all Chardonnay samples. Judging on the standardized coefficients obtained (**Table 7**), separation of

Table 6. Classification Matrix of the Original SLDA Model and Cross-Validation for Istrian Malvasia (MAL), Muscat Blanc (MB) and Rose Muscat of Poreč (RMP) Grape Marc Distillates

	original category membership		predicted category membership			
			MAL	MB	MRP	total
original model[a]	count	MAL	5	0	0	5
		MB	0	5	0	5
		RMP	0	0	5	5
	%	MAL	100.0	0.0	0.0	100.0
		MB	0.0	100.0	0.0	100.0
		RMP	0.0	0.0	100.0	100.0
cross-validated[b]	count	MAL	5	0	0	5
		MB	0	5	0	5
		RMP	0	0	5	5
	%	MAL	100.0	0.0	0.0	100.0
		MB	0.0	100.0	0.0	100.0
		RMP	0.0	0.0	100.0	100.0

[a] Average percentage of correct classification of 100.0%. [b] Average percentage of correct prediction of 100.0%.
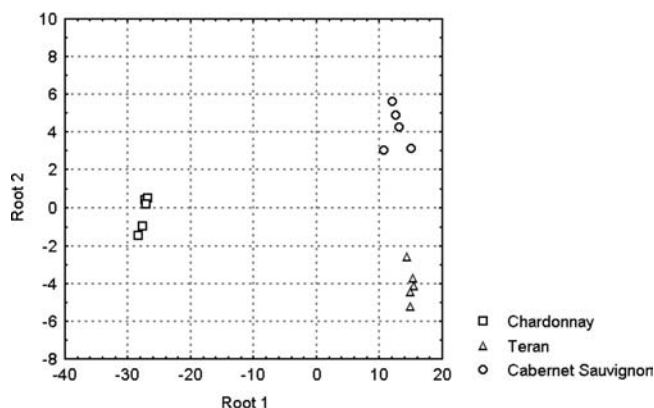

Figure 2. Projection of Chardonnay, Teran, and Cabernet Sauvignon grape marc distillate samples, classified according to varietal origin, along the directions of two discriminant functions.

Table 7. Standardized Coefficients of the Variables Contributed to the Differentiation of Chardonnay, Teran and Cabernet Sauvignon Grape Marc Distillates Obtained by SLDA

variable	root 1	root 2
β-ionone	6.6329	0.0828
cis-linalool-furan-oxide	−9.7304	−1.5587
α-cadinol	−9.0791	−4.3385
α-ylangene	4.7050	−17.3346
manoyloxide	4.0627	0.9030
vitispirane B	2.8739	0.5892
isoledene	−0.9830	15.7639
α-muurolene	−0.4608	4.6212

Chardonnay from red grape distillates along the first function depended on the concentrations of the majority of compounds included in the model, except for isoledene and α-muurolene. When *cis*-linalool-furan-oxide, α-cadinol, and α-ylangene became part of the model, the percentage of overall correctly classified samples increased to 86.7% (100% of Cabernet samples), while including manoyloxide, vitispirane B, and isoledene resulted in the correct classification of 100% of all distillate samples. Differentiation of Teran and Cabernet Sauvignon along the direction of the second

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Table 8. Classification Matrix of the Original SLDA Model and Cross-Validation for Chardonnay (CH), Teran (TE), and Cabernet Sauvignon (CS) Grape Marc Distillates

	original category membership		predicted category membership			
			CH	TE	CS	total
original model[a]	count	CH	5	0	0	5
		TE	0	5	0	5
		CS	0	0	5	5
	%	CH	100.0	0.0	0.0	100.0
		TE	0.0	100.0	0.0	100.0
		CS	0.0	0.0	100.0	100.0
cross-validated[b]	count	CH	4	0	1	5
		TE	0	5	0	5
		CS	0	0	5	5
	%	CH	80.0	0.0	20.0	100.0
		TE	0.0	100.0	0.0	100.0
		CS	0.0	0.0	100.0	100.0

[a] Average percentage of correct classification of 100.0%. [b] Average percentage of correct prediction of 93.3%.

function seems to mainly be due to differences in the concentrations of α-ylangene, isoledene, and α-cadinol (**Table 7**). By conducting cross-validation procedure, a correct prediction of 93.3% of all samples was obtained (**Table 8**).

The results of the present investigation showed that marc distillates made from Istrian Malvasia, Chardonnay, Muscat Blanc, Rose Muscat of Poreč, Teran, and Cabernet Sauvignon grape varieties are characterized by different varietal aroma composition. Two Muscat distillates exhibited profiles significantly distinct from those of other varieties, with a considerably larger number and concentration of monoterpenes, which sort them in the group of highly aromatic distillates with complex Muscat aroma. Istrian Malvasia showed less complex composition with still a notable number and concentration of major monoterpenols which may turn out to be the important feature in contrast to Chardonnay, Teran, and Cabernet Sauvignon, which exhibited poorer monoterpene profiles. Teran distillates contained elevated sesquiterpene concentrations. Final considerations regarding Cabernet Sauvignon varietal aroma are not possible at this stage of the investigation because the method used did not allow important methoxypyrazines to be identified and quantified.

It was presumed that the information on the specific varietal aroma potential of the investigated varieties could be very useful in focusing the efforts of producers in developing targeted technologies for the production of monovarietal marc distillates, all in order to improve their specific quality. Since it is known that today consumers prefer products with information about specific organoleptic attributes related to varietal origin, this data could be used in elaborating and attributing a certain added value to these products.

Many varietal aroma compounds emerged as potential indicators of varietal origin. This especially refers to monoterpenes, many of which were found unique for Muscat Blanc and Rose Muscat of Poreč distillates. α-Terpineol exhibited the greatest discriminating power in chemometric elaboration, meaning that it is possible to achieve efficient differentiation of some varieties only by focusing the analysis on this particular compound. Stepwise linear discriminant analysis also extracted various sesquiterpenes and C_{13}-norisoprenoids as important, especially for differentiation of distillates with poorer varietal aroma

potential. This fact, together with notable sesquiterpene concentrations found in Teran distillates, suggest that these compounds were generally unjustifiably neglected in previous research in this field. It was assumed that this approach could be analogously applied for the characterization and differentiation of marc distillates produced from the same or related grape varieties in other geographical areas worldwide.

In the future, further research in this area should address the identification and quantification of methoxypyrazines, and the study of the influence of harvest year, row material (grape marc) quality, and different postdistillation treatments, such as redistillation, chill filtration, clarification, and aging, on varietal aroma. Our group is currently working on such topics.

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