



Influence of grape composition and origin, yeast strain and spontaneous fermentation on aroma profile of Corvina and Corvinone wines

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ARTICLE INFO

Keywords:

Aroma compounds
Red wines
Grape origins
Saccharomyces cerevisiae
Spontaneous fermentation

ABSTRACT

Volatile composition and sensory properties of Corvina and Corvinone red wines in relationship to grape origin, yeast strain and inoculated vs. spontaneous fermentation were investigated. Experimental wines were produced using freshly harvested grapes of the two varieties coming from two different areas. The results indicated that, by affecting grape composition, grape origin had a primary impact on wine aroma chemical and sensory properties. From a chemical point of view, this effect was associated not only with grape-derived compounds but also to some extent with fermentation-derived esters. Yeast strains influence was mostly associated with higher alcohols and certain esters, whereas the main characteristic of spontaneous fermentation was increased concentration of ethyl acetate. Sensory analysis confirmed the greater impact of grape origin compared to yeast strain, indicating clusters of odor similarities which were mostly associated with variations in the content of ethyl esters, C₆ alcohols, and norisoprenoids in Corvina and of norisoprenoids, cyclic terpenes, acetate esters, and ethyl acetate in Corvinone, respectively.

1. Introduction

Wine aroma arises from a complex combination of chemical and biochemical transformations taking place during wine production. Depending on their concentration, only a portion of the more than 800 volatile identified compounds is actually contributing to wine perceived aroma (Ferreira, 2010). Many of these compounds, in particular terpenoids and norisoprenoids, originate in the grape, and in the majority of the *Vitis vinifera* varieties they occur mostly in the form of non-volatile, odourless precursors (Crupi et al., 2020; Parker, Capone, Francis, & Herderich, 2018). Other compounds, such as esters, although present in small amounts in grapes, are produced in large quantities during fermentation by yeast enzymatic activities (Ugliano & Henschke, 2009). Complex relationships exist between these two main sources of volatile compounds, so that, for example, volatiles bound to precursors are in part released by yeast during fermentation, and some of the released compounds can be also further transformed by the yeast itself (Ugliano & Henschke, 2009).

In the context of wine production, connection between wine sensory characteristics, especially aroma, and geographical origin, is of particular relevance (van Leeuwen et al., 2004). Specific geographical origins associated with sensory features, are perceived as an added value by

consumers (Cross, Plantinga, & Stavins, 2011). Occurrence of certain aroma compounds and precursors can be deeply influenced by vineyard characteristics and geographical location (Slaghenaufi, Guardini, Tedeschi, & Ugliano, 2019). A first question that arises is to what extent these variations are transferred into wine to a degree that can be sensorially relevant. Moreover, recent investigations indicated that grape composition can also affect the biosynthesis of volatile compounds that are thought to be primarily of fermentative origin (Robinson, Boss, Heymann, Solomon, & Trengove, 2011; Antalick, Šuklje, et al., 2015). In consideration of the central role of fermentation in the process of wine aroma development, the yeast conducting the fermentation is considered of primary importance for developing specific aroma profiles (Bellon, Yang, Day, Inglis, & Chambers, 2019; Bisson & Karpel, 2010; Borneman et al. 2011). In the context of the above-mentioned importance of wine geographical origin, it becomes of increasing relevance to quantify the actual contribution of inoculation strategies based on commercial yeast strains to the expression of aroma characters in wine. In addition, in recent years there has been a growing interest towards the possible contribution of spontaneous fermentations to the expression of distinctive aroma features linked to grape geographical origin and expression of terroir attributes (Nicola et al., 2016).

The aim of this work was to evaluate the influence and the relative

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<https://doi.org/10.1016/j.lwt.2021.111120>

Received 8 October 2020; Received in revised form 10 February 2021; Accepted 11 February 2021

Available online 15 February 2021

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Table 1
Enological parameters of Corvina and Corvinone musts at crush.

	GLUCOSE + FRUCTOSE (g/L)		TOTAL ACIDITY (g/L)		pH		PAN ^a (mg/L)		AMMONIA (mg/L)		YAN ^b (mg/L)	
	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
Area 1 Corvina	199.6 ab	4.1	3.9 b	0.31	3.13 ab	0.03	92.1 b	7.3	56.1 b	7.5	138.2 b	12.9
Area 2 Corvina	203.1 ab	9.4	3.56 a	0.2	3.1 a	0.05	84.7 a	3.8	48.8 a	3.7	124.8 a	6.5
Area 1 Corvinone	192.5 a	12.9	4.26 c	0.19	3.17 b	0.03	117.2 d	6.7	77.2 d	6.1	180.7 d	11.4
Area 2 Corvinone	207.7 b	7.5	3.93 b	0.25	3.18 b	0.03	103.6 c	5.9	66.7 c	5.8	158.5 c	10.2

^a PAN: Primary Amino Nitrogen.

^b YAN: Yeast Assimilable Nitrogen. Different letters denote statistically significant difference as obtained by ANOVA ($\alpha = 0.05$) with post-hoc Tukey test.

contribution of grape origin, different commercial yeast strains, and spontaneous fermentation on aroma profile and sensory characteristics of wines from Corvina and Corvinone grapes, the two major varieties of the Valpolicella region. These varieties are employed for all Valpolicella wines including the well-known Amarone della Valpolicella produced with withered grapes. In this study experimental wines of the Valpolicella Classico type were considered, so that grapes were not submitted to any post-harvest withering.

2. Materials and methods

2.1. Grape samples and vinification

Wines were produced with either Corvina (*Vitis vinifera* L. cv. Corvina) or Corvinone (*Vitis vinifera*, L. cv. Corvinone), the two main grape varieties of the Valpolicella appellation. Grapes were harvested in mid-September 2018 in vineyards belonging to the same winery and located in two sub-regions within the Valpolicella area, locally referred to as Valpolicella Orientale (Area 1) and Valpolicella Classica (Area 2). Specifically, for Area 1, grapes were obtained from one vineyard parcel located in the town of Mezzane (45°30'36.7"N 11°08'02.7"E). In the case of Area 2, two vineyard parcels were considered, located at a distance of approximately 5 km from each other, in the towns of San Pietro in Cariano (45°30'40.5"N 10°54'58.6"E) and San Giorgio in Valpolicella (45°32'16.6"N 10°51'19.4"E), respectively. The grapes of these two parcels were pooled together to have a single vinification batch, as regularly done in the winery. All vineyard had the same clone-rootstock combination. Grapes were hand-harvested from the 17th of September to the October 1, 2018. Grapes were destemmed and the berries randomized to obtain batches of 20 Kg each. From each batch, 800 g were taken, hand crushed with 80 mg of potassium metabisulphite and put into 1.5 L glass vessel. Analytical parameters of the musts are provided in Table 1. Fermentations were carried out in duplicate with four different commercial yeasts, *Saccharomyces cerevisiae* x *Saccharomyces kudriavzevi* AWRI 1503 (AB Mauri, Camellia, Australia), labelled as Yeast 1, *Saccharomyces cerevisiae* AWRI 796 (AB Mauri, Camellia, Australia), labelled as Yeast 2, *Saccharomyces cerevisiae* Zymaflore® XPURE (Laffort, Floirac, France), labelled as Yeast 3, *Saccharomyces cerevisiae* Zinfandel (Vason, Verona, Italy), labelled as Yeast 4. Active dry yeast were rehydrated in water at 37 °C for 15 min, then 1.6 mL of each culture (100 g/L) was used to inoculate individual grape batches. An additional experimental modality was also prepared, consisting of a spontaneous fermentation (Spontaneous). For this modality, no potassium metabisulphite was added at berry crushing. Fermentations were carried out at 22 ± 1 °C, with cap being broken twice a day by gently pressing it down with a steel plunger and density and temperature monitored daily. Upon completion of alcoholic fermentation (glucose-fructose < 2 g/L), wines were pressed using a 10 L basket press and supplemented with potassium metabisulphite until a final free SO₂ concentration of 25 mg/L. Wines were then clarified by centrifugation at 4500 rpm for 15 min at 5 °C using a Beckman-Coulter Avant J-25 centrifuge equipped with a J-Lite 10.5000 fixed angle rotor (Beckman-Coulter, Brea, CA) and bottled in 330 mL glass bottles with crown caps, with free SO₂ concentration of 25 mg/L.

2.2. Standard enological analyses

Glucose-fructose, ammonia, primary amino nitrogen (PAN), acetic acid, and total acidity were analysed using a Biosystems Y15 multi-parametric analyser (Sinatech, Fermo, Italy). YAN (yeast assimilable nitrogen) was obtained as the sum of PAN and ammonia. For each parameter, a specific kit (Sinatech, Fermo, Italy) was used. Ethanol was analysed with an Alcozyler dma 4500 (Anton Paar, Graz, Austria).

2.3. Volatile compounds analysis

The volatile compounds analysed in the study, with the analytical performances of the relevant method, are indicated in [Supplementary 1](#).

2.3.1. SPE-GC-MS

For quantification of alcohols, esters, fatty acids, benzenoids, and other compounds, SPE extraction followed by GC-MS analysis was used, following the procedure described by [Slaghenaufi et al. \(2019\)](#). One-hundred µL of 2-octanol (internal standard 4.2 mg/L in ethanol) were added to samples prepared with 50 mL of wine and diluted with 50 mL of deionized water. Samples were loaded on a BOND ELUT-ENV, SPE cartridge, (Agilent Technologies, USA) previously activated with 20 mL of dichloromethane, 20 mL of methanol and equilibrated with 20 mL of water. After sample loading, the cartridges were washed with 15 mL of water. Free volatile compounds were eluted with 10 mL of dichloromethane and then concentrated under gentle nitrogen stream to 200 µL prior to GC injection. GC-MS analysis was carried out on an HP 7890A (Agilent Technologies) gas chromatograph coupled to a 5977B quadrupole mass spectrometer, equipped with a Gerstel MPS3 auto sampler (Müllheim/Ruhr, Germany). Separation was performed using a DB-WAX UI capillary column (30 m × 0.25 mm i. d., 0.25 µm film thickness, Agilent Technologies) and helium as carrier gas at 1.2 mL/min of constant flow rate. GC oven was programmed as follow: started at 40 °C for 3 min, raised to 230 °C at 4 °C/min and maintained for 20 min. Mass spectrometer operated in electron ionization (EI) at 70 eV with ion source temperature at 250 °C and quadrupole temperature at 150 °C. Mass spectra were acquired in synchronous Scan and SIM mode. A calibration curve was prepared for each analyte using seven concentration points and three replicate solutions per point in model wine (12% v/v ethanol, 3.5 g/L tartaric acid, pH 3.5). One-hundred µL of 2-octanol (internal standard, 4.2 mg/L in ethanol) were added to each calibration solution, which was then submitted to SPE extraction and GC-MS analysis as described for the samples. Calibration curves were obtained using Chemstation software (Agilent Technologies, Inc.) by linear regression, plotting the response ratio (analyte peak area divided by internal standard peak area) against concentration ratio (added analyte concentration divided by internal standard concentration).

2.3.2. HS-SPME-GC-MS

For quantification of terpenes, norisoprenoids, ethyl acetate, and methyl salicylate, SPME extraction followed by GC-MS analysis was used, following the procedure described by [Slaghenaufi & Ugliano. \(2018\)](#). Five µL of 2-octanol (internal standard 4.2 mg/L in Ethanol) are added to 5 mL of wine diluted with 5 mL of deionized water in a 20 mL

Table 2
Enological parameters of wines at the end of alcoholic fermentation.

		Yeast 1		Yeast 2		Yeast 3		Yeast 4		Spontaneous	
		mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
Area 1 Corvina	Total acidity (g/L)	6.1 d	0.1	8.0 a	0	6.5 c	0.1	5.9 d	0	7.6 b	0.1
	pH	3.05 a	0.01	2.96 b	0.01	3.05 a	0.01	3.01 ab	0.01	2.99 ab	0.03
	Acetic acid (g/L)	0.16 a	0.08	0.32 a	0.04	0.28 a	0.08	0.12 a	0.03	0.20 a	0.05
	Ethanol (% v/v)	11.94 a	0.06	12.01 a	0.04	11.96 a	0.21	12.02 a	0.06	11.96 a	0.11
Area 2 Corvina	Total acidity (g/L)	6.5 c	0.2	8.3 a	0.1	7.6 b	0.1	7.1 b	0.1	8.3 a	0.1
	pH	2.88 a	0.01	2.92 a	0.03	2.83 ab	0.04	2.91 a	0.01	2.78 b	0
	Acetic acid (g/L)	0.16 a	0.03	0.24 a	0.10	0.26 a	0.06	0.14 a	0.01	0.18 a	0.03
	Ethanol (% v/v)	11.74 a	0.01	12.01 a	0.04	11.87	0.05	11.93 a	0.16	11.94 a	0.04
Area 1 Corvinone	Total acidity (g/L)	6.7 b	0.1	7.7 a	0.2	5.8 b	0.1	6.1 c	0.1	7.3 a	0.1
	pH	3.12 b	0.02	3.03 c	0.04	3.14 a	0.01	3.06 bc	0.01	3.05 bc	0.04
	Acetic acid (g/L)	0.27 a	0.11	0.22 a	0.08	0.16 a	0.04	0.14 a	0.04	0.26 a	0.09
	Ethanol (% v/v)	11.95 a	0.06	12.03 a	0.03	11.97 a	0.25	12.09 a	0.13	11.96 a	0.13
Area 2 Corvinone	Total acidity (g/L)	6.4 d	0.1	9.0 a	0	6.8 c	0.1	6.5 cd	0.1	7.4 b	0.1
	pH	2.95 a	0.07	2.91 a	0.01	2.97 a	0.03	3.01 a	0	2.9 a	0.01
	Acetic acid (g/L)	0.20 a	0.03	0.18 ab	0.00	0.22 a	0.01	0.12 b	0.03	0.18 ab	0.01
	Ethanol (% v/v)	12.06 c	0.06	12.83 a	0.06	11.72 d	0.06	12.48 b	0.04	11.97 c	0.08

Within the same row values with different letters indicate statistically significant differences according to ANOVA analysis ($p < 0.05$).

glass vial. Three g of NaCl were added prior to GC-MS analysis. Samples were equilibrated for 1 min at 40 °C. Subsequently, SPME extraction was performed using a 50/30 μm divinylbenzene-carboxen-polydimethylsiloxane (DVB/CAR/PDMS) fiber (Supelco, Bellefonte, PA, USA) exposed to sample headspace for 60 min at 40 °C. Desorption was done into the injector port at 250 °C for 5 min in splitless mode. GC-MS analysis were performed as described in the previous section.

2.4. Sorting task

Sorting task was carried out to find wines similitudes or differences as described by Alegre et al. (2017) with slight differences. Twelve judges participated in the study. All were wine-science researchers, university teaching staff or oenology students, involved in winemaking or wine evaluation. One hour before the tests, samples were removed from the 16 °C room and 20 mL were poured in wine glasses labelled with 3-digit random codes and covered by plastic Petri dishes; all samples were served at room temperature, and glasses were randomized for each panellist. Panellists were asked to sort the wines into groups based on aroma similarities. They could make as many groups as they wished. Two separate sessions were carried out, one for Corvina wines and one for Corvinone wines. Twenty Corvina wines and twenty Corvinone wines were submitted to sorting tasks (ten different wines for each variety with their biological replicates). No time limit was set for the test.

2.5. Statistical analyses

Principal Component Analysis (PCA), one-way ANOVA ($\alpha = 0.05$), two-way ANOVA ($\alpha = 0.05$), Kruskal-Wallis and Mann-Whitney tests of chemical data have been performed using XLSTAT 2017. Hierarchical Cluster Analysis (HCA) as described by Alegre et al. (2017), have been performed using XLSTAT 2017 (Addinsoft SARL, Paris, France).

3. Results and discussion

3.1. Main enological parameters of grapes and wines

Enological parameters of musts at crush are given in Table 1. Glucose and fructose content varied to a minor extent between vineyard sites, with Area 2 grapes showing slightly higher content than Area 1. Total acidity varied significantly across grape batches, and Corvina grapes showed lower total acidity values than Corvinone. pH was similar in all musts, with values comprised between 3.1 and 3.18. YAN was higher in Corvinone grapes compared to Corvina, with smaller differences

occurring within the same variety. All these data reflect the natural variation in grape composition arising from pedoclimatic differences across the two zones. Fermentation kinetics for the different treatments are shown in Supplementary 2, indicating that difference across commercial yeast strains were relatively small, whereas spontaneous fermentations showed generally a slower start in all grape batches compared to inoculated fermentations. Area 2 generally gave faster fermentations. Enological parameters of wines are shown in Table 2, with the relevant ANOVA analysis presented in Supplementary 3. Ethanol was not affected by yeast but by grape origin in Corvinone, with Area 2 Corvinone wines showing higher content, in agreement with the higher glucose and fructose contents. Total acidity showed significant differences according to yeasts and the interaction between yeasts and areas, in particular Yeast 2 and Spontaneous fermentation shows higher total acidity. Instead, pH of both varieties depended for both varieties on grape area and area*yeast interaction but never on yeasts alone. Acetic acid content was, in all wines, lower than the legal limit as well as its odor threshold (0.48 g/L), with Yeast 4 showing generally lower levels than the others. Overall, the results of the analyses of the main enological parameters did not show clear patterns that could be ascribed to either grape variety or origin.

3.2. Wine volatile composition

A total of 61 volatile compounds were analysed in different wines (Tables 3–6). In the case of Corvina (Tables 3 and 4), 36 volatile compounds were found to significantly discriminate wines according to grape origin (Supplementary 4) ($\alpha = 0.05$) including some C₆ alcohols, some esters and acids, as well as the majority of the terpenes, norisoprenoids, and benzenoids analysed. Twenty-two volatile compounds showed statistically significant differences due to yeast strain/spontaneous fermentation, including the majority of alcohols, esters, acids. Two-way ANOVA indicated that 24 volatile compounds were significantly different, including in this case also some norisoprenoids. In the case of Corvinone wines (Tables 5 and 6), 30 volatile compounds were significantly different according to grape area (Supplementary 4), including C₆ alcohols, several acetate esters, and the majority of terpenes, norisoprenoids, and benzenoids. Twenty volatile compounds showed significant differences linked to the yeast used, and these included alcohols, some esters (but among acetate esters only ethyl acetate), fatty acids, and only a few among the terpenes, norisoprenoids, and benzenoids analysed. Two-way ANOVA indicated that 26 volatile compounds were significantly different according to the Yeast*Area interaction (Supplementary 4).

PCA applied to the entire volatiles dataset indicated that, in the case

Table 3
Concentration ($\mu\text{g/L}$) and standard deviation ($\pm\mu\text{g/L}$) of volatile compounds of Area 1 Corvina wines.

	Yeast 1		Yeast 2		Yeast 3		Yeast 4		Spontaneous	
	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
Alcohols										
1-Butanol	122.35	18.89	30.96	4.21	96.31	14.88	79.65	3.44	95.34	15.92
2-Butanol	4754.15	516.08	5066.65	785.21	7436.22	591.31	3367.31	443.36	6183.53	900.13
1-Pentanol	54.19	2.23	65.98	4.52	53.63	2.98	58.06	2.44	50.14	2.54
Isoamyl alcohol	200766.90	4624.69	127612.44	1863.95	207312.00	3583.44	128934.53	6645.99	165303.54	1830.17
Phenethyl Alcohol	17843.47	3650.70	8463.27	1437.53	16316.05	3379.22	10618.33	40.72	12538.69	864.87
Methionol	568.05	48.40	196.93	39.48	442.90	65.83	592.42	61.45	236.33	49.74
C₆ alcohols										
1-Hexanol	1908.95	13.36	1999.98	288.02	1871.12	79.62	1823.62	56.01	1964.68	183.38
trans-3-Hexen-1-ol	24.99	0.66	27.69	3.67	24.80	0.16	23.01	0.94	15.24	2.03
cis-3-Hexen-1-ol	342.05	16.83	316.73	7.66	381.92	33.31	359.59	17.05	334.87	58.53
cis-2-Hexen-1-ol	13.53	0.16	12.74	0.51	13.14	0.23	12.99	0.52	12.85	0.11
Acetate esters										
Isoamyl acetate	689.11	20.22	776.42	112.93	592.05	87.98	375.62	139.13	569.68	70.32
n-Hexyl acetate	10.18	0.18	8.36	0.45	5.13	0.33	7.17	2.31	6.61	0.40
2-Phenethyl acetate	31.85	0.34	22.80	2.32	22.62	0.78	20.79	0.95	28.15	3.90
Ethyl acetate	37166.75	4901.37	61875.72	8240.78	38044.55	9050.34	26928.97	8510.64	79875.61	13114.12
Branched-chain fatty acids ethyl esters										
Ethyl 2-methyl butanoate	1.98	0.01	2.85	0.12	2.93	0.23	2.02	0.09	2.36	0.27
Ethyl 3-methyl butanoate	1.17	0.10	3.49	0.33	4.82	0.14	1.43	0.07	2.75	0.11
Ethyl fatty acids esters										
Ethyl butanoate	165.57	14.18	227.62	42.04	149.66	32.86	147.63	31.64	146.45	7.12
Ethyl hexanoate	591.46	1.31	643.46	101.14	617.57	173.01	493.77	116.29	511.05	82.22
Ethyl octanoate	345.22	6.14	373.28	58.29	395.62	72.66	257.28	70.38	258.71	46.95
Ethyl decanoate	78.80	4.62	77.55	5.59	88.73	23.49	65.04	21.06	50.91	6.13
Other esters										
Ethyl 3-hydroxybutanoate	256.18	30.84	267.01	32.01	386.95	30.61	163.26	17.25	273.50	24.20
Ethyl 2-hydroxyhexanoate	0.38	0.03	1.01	0.14	1.36	0.10	0.39	0.01	1.02	0.07
Fatty acids										
3-Methylbutanoic acid	408.47	52.87	432.76	55.32	486.09	48.02	364.98	0.82	289.17	31.23
Hexanoic acid	4032.16	452.00	4942.90	813.38	3906.8	697.68	3189.58	569.6	3531.62	590.9
Octanoic acid	6361.54	391.43	6512.38	1130.3	6070.5	774.51	5393.54	498.3	5273.32	526.5
Terpenoids										
cis-Linalooloxide	0.17	0.02	0.50	0.00	0.24	0.00	0.35	0.04	0.57	0.04
trans-Linalooloxide	0.46	0.00	0.53	0.04	0.48	0.03	0.56	0.01	0.51	0.08
Linalool	6.12	0.47	6.56	0.74	8.12	1.24	6.96	0.11	7.07	0.54
Geraniol	4.75	0.72	5.04	0.03	7.43	0.13	14.62	2.55	4.51	0.05
β -Citronellol	15.52	1.73	13.04	1.40	13.36	0.30	11.34	0.55	15.35	1.22
α -Terpineol	1.53	0.04	2.04	0.28	2.24	0.23	1.56	0.13	2.22	0.13
3-Carene	0.08	0.01	0.09	0.01	0.07	0.01	0.07	0.01	0.08	0.01
α -Phellandrene	2.56	0.30	2.72	0.23	3.37	0.42	3.53	0.16	2.68	0.23
α -Terpinen	0.05	0.01	0.06	0.00	0.06	0.00	0.07	0.00	0.07	0.01
β -Myrcene	2.84	0.37	3.55	0.03	4.38	0.54	5.23	0.55	3.48	0.30
Limonene	0.29	0.03	0.32	0.04	0.43	0.02	0.46	0.08	0.37	0.04
1,8-Cineol	0.08	0.01	0.00	0.00	0.06	0.00	0.07	0.01	0.06	0.01
p-Cymene	0.15	0.02	0.18	0.01	0.15	0.00	0.16	0.01	0.16	0.01
Terpinolene	0.19	0.02	0.21	0.01	0.28	0.02	0.33	0.04	0.26	0.03
Terpinen-1-ol	0.17	0.01	0.16	0.01	0.14	0.01	0.15	0.02	0.13	0.01
Terpinen-4-ol	0.00	0.00	0.00	0.00	0.15	0.02	0.20	0.02	0.15	0.01
Nerol	1.20	0.08	1.23	0.09	1.07	0.08	0.97	0.01	0.97	0.02
Norisoprenoids										
β -Damascenone	1.39	0.07	1.54	0.02	1.82	0.24	1.73	0.08	1.42	0.11
3-Oxo- α -ionol	1.29	0.20	1.19	0.04	1.17	0.04	1.49	0.01	1.64	0.09
3-Hydroxy- β -damascone	0.12	0.01	0.08	0.00	0.13	0.00	0.12	0.01	0.10	0.01
Vitispirane	0.11	0.01	0.30	0.00	0.24	0.01	0.23	0.01	0.24	0.01
TPB	0.00	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00
TDN	0.07	0.00	0.09	0.01	0.08	0.01	0.07	0.00	0.08	0.01
Benzenoids and others										
Benzyl Alcohol	139.11	7.96	98.13	10.53	146.93	11.10	150.57	16.30	167.28	10.50
Vanillin	6.00	0.47	4.63	0.15	4.64	0.45	4.78	0.21	3.75	0.08
Vanillyl alcohol	4.40	3.51	9.89m	1.43	6.25	3.83	4.94	0.58	7.41	0.54
Ethyl vanillate	93.62	12.08	119.86	18.16	93.41	9.06	99.93	5.56	100.78	1.36
Methyl vanillate	6.04	0.25	10.18	1.50	6.08	0.17	6.01	0.66	6.78	0.16
Benzaldehyde	14.44	0.22	14.21	0.21	14.56	0.13	14.51	0.06	14.49	0.06
Eugenol	5.95	0.03	4.03	0.57	5.67	0.08	5.90	0.51	5.79	0.45
Methyl salicylate	0.45	0.01	0.56	0.06	0.46	0.06	0.49	0.08	0.48	0.06
γ -Decalactone	3.12	0.00	2.37	0.06	2.94	0.21	2.65	0.52	3.44	0.40
δ -Decalactone	17.45	1.85	18.75	1.93	16.83	2.71	16.95	1.56	14.72	1.92
2,6-Dimethoxyphenol	9.58	1.38	7.79	0.81	10.02	0.73	8.07	0.16	9.38	1.71
Furfural	1.84	0.06	1.86	0.18	2.24	0.10	0.94	0.64	0.48	0.01

Table 4
Concentration and standard deviation ($\mu\text{g/L}$) of volatile compounds of Area 2 Corvina wines.

	Yeast 1		Yeast 2		Yeast 3		Yeast 4		Spontaneous	
	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
Alcohols										
1-Butanol	129.27	3.54	86.60	1.80	79.28	3.44	82.03	6.55	79.12	8.50
2-Butanol	4710.82	185.98	5267.86	1041.31	6645.56	346.46	3773.92	214.89	6147.55	2.17
1-Pentanol	48.14	2.04	47.04	5.08	42.66	1.31	48.29	0.10	41.97	1.57
Isoamyl alcohol	199191.56	3845.83	191812.01	6408.83	195398.99	11683.16	153611.39	1270.93	178727.20	6756.43
Phenethyl alcohol	17622.72	297.71	13270.72	358.63	14853.93	266.58	12553.71	162.08	13314.45	43.07
Methionol	423.47	10.47	268.10	45.16	257.37	19.83	544.67	58.83	251.46	7.93
C₆ alcohols										
1-Hexanol	2456.77	84.71	2174.94	46.34	2429.56	239.06	2318.01	41.12	2534.44	241.43
<i>trans</i> -3-Hexen-1-ol	18.06	1.89	16.14	0.74	18.02	2.42	19.10	0.57	12.79	1.64
<i>cis</i> -3-Hexen-1-ol	240.69	10.54	256.92	38.19	292.78	10.30	238.84	13.80	220.40	35.12
<i>cis</i> -2-Hexen-1-ol	15.23	0.00	13.29	0.42	13.66	0.52	13.27	0.44	13.48	0.45
Acetate esters										
Isoamyl acetate	543.38	71.59	1564.80	96.36	761.32	26.33	393.45	9.62	731.98	35.77
n-Hexyl acetate	10.36	0.23	30.48	0.37	10.87	0.96	10.18	0.21	13.37	0.25
2-Phenethyl acetate	36.46	3.05	42.02	1.48	31.87	0.11	24.64	1.91	49.46	0.56
Ethyl acetate	33001.87	862.65	49384.07	2097.55	38248.41	1886.17	24175.72	265.69	76531.61	138.71
Branched-chain fatty acids ethyl esters										
Ethyl 2-methyl butanoate	3.10	0.37	3.70	0.04	4.47	0.16	3.03	0.28	2.39	0.12
Ethyl 3-methyl butanoate	2.91	0.08	6.46	0.54	6.52	0.81	2.16	0.17	2.28	0.18
Ethyl fatty acids esters										
Ethyl butanoate	130.86	7.23	245.09	35.56	113.79	0.76	126.83	6.82	131.91	14.96
Ethyl hexanoate	488.08	39.79	931.03	81.33	454.02	53.79	439.14	18.87	509.42	57.62
Ethyl octanoate	241.98	37.13	426.33	26.90	200.43	54.15	163.07	43.46	206.46	34.39
Ethyl decanoate	43.08	6.93	80.22	3.13	43.34	6.68	34.61	7.85	37.69	6.36
Other esters										
Ethyl 3-hydroxybutanoate	230.32	11.53	248.23	14.96	264.29	19.76	160.48	4.94	249.89	11.29
Ethyl 2-hydroxyhexanoate	0.63	0.10	1.27	0.23	1.92	0.01	0.55	0.04	0.76	0.01
Fatty acids										
3-Methylbutanoic acid	452.51	73.32	486.57	23.85	574.64	10.46	427.19	14.04	283.83	8.78
Hexanoic acid	3167.93	159.97	4523.44	737.75	2829.67	278.07	2713.65	108.76	3114.00	177.93
Octanoic acid	5303.09	10.22	6587.47	352.25	4587.83	401.25	4564.29	6.55	4832.87	383.63
Terpenoids										
<i>cis</i> -Linalooloxide	0.57	0.04	0.39	0.06	0.56	0.06	0.47	0.04	0.70	0.05
<i>trans</i> -Linalooloxide	0.56	0.03	0.69	0.06	0.53	0.03	0.67	0.01	0.71	0.03
Linalool	12.11	0.65	13.76	0.15	12.49	1.01	13.35	0.74	13.91	0.74
Geraniol	3.49	0.36	7.16	0.07	4.94	0.04	7.60	0.81	6.61	0.57
β -Citronellol	10.71	0.06	9.47	0.96	10.47	0.74	8.35	0.08	12.02	0.16
α -Terpineol	3.29	0.19	4.03	0.03	4.06	0.25	3.75	0.25	3.92	0.44
3-Carene	0.06	0.01	0.07	0.01	0.09	0.00	0.06	0.00	0.06	0.01
α -Phellandrene	5.12	0.75	5.96	0.16	5.95	0.72	7.20	1.02	5.19	0.02
α -Terpinen	0.11	0.02	0.13	0.01	0.12	0.01	0.16	0.01	0.16	0.01
β -Myrcene	6.65	0.98	7.74	0.22	7.73	0.93	9.35	1.33	6.74	0.02
Limonene	1.06	0.10	1.17	0.14	1.12	0.25	1.49	0.13	1.26	0.01
1,8-Cineol	0.10	0.01	0.12	0.01	0.12	0.02	0.19	0.02	0.13	0.02
<i>p</i> -Cymene	0.20	0.01	0.24	0.02	0.23	0.01	0.22	0.04	0.25	0.04
Terpinolene	0.56	0.07	0.73	0.03	0.64	0.04	0.68	0.06	0.80	0.01
Terpinen-1-ol	0.12	0.01	0.13	0.01	0.12	0.01	0.09	0.04	0.12	0.01
Terpinen-4-ol	0.21	0.02	0.23	0.01	0.44	0.06	0.54	0.19	0.59	0.09
Nerol	0.82	0.16	1.12	0.13	0.52	0.06	0.65	0.06	0.63	0.04
Norisoprenoids										
β -Damascenone	1.92	0.11	1.29	0.09	1.60	0.23	1.78	0.27	1.56	0.06
3-Oxo- α -ionol	0.91	0.01	1.05	0.00	0.85	0.12	0.96	0.03	1.10	0.16
3-Hydroxy- β -damascone	0.18	0.01	0.07	0.00	0.08	0.00	0.07	0.01	0.08	0.01
Vitispirane	1.20	0.04	1.24	0.25	1.74	0.13	1.68	0.12	2.17	0.05
TPB	0.02	0.00	0.02	0.00	0.03	0.00	0.03	0.01	0.04	0.01
TDN	0.31	0.04	0.30	0.02	0.41	0.03	0.36	0.01	0.49	0.00
Benzenoids and others										
Benzyl Alcohol	104.56	16.83	104.99	4.09	101.86	0.45	113.40	5.51	108.87	5.61
Vanillin	4.63	0.42	3.79	0.60	3.67	0.28	4.07	0.08	3.66	0.12
Vanillyl alcohol	4.98	0.95	3.22	2.66	4.61	0.17	4.82	0.80	5.73	0.81
Ethyl vanillate	96.43	1.82	95.34	0.71	91.83	0.77	92.87	2.24	92.95	7.93
Methyl vanillate	8.46	0.78	7.90	0.11	7.94	0.23	7.96	0.39	8.00	0.15
Benzaldehyde	16.57	2.72	14.77	0.05	17.22	0.38	14.73	0.34	14.70	0.12
Eugenol	0.92	0.02	1.21	0.01	0.97	0.06	1.16	0.07	0.88	0.14
Methyl salicylate	3.06	0.23	3.06	0.18	2.98	0.11	3.01	0.16	3.17	0.09
γ -Decalactone	5.16	0.75	3.60	0.36	3.89	0.55	3.86	0.35	3.86	0.25
δ -Decalactone	15.66	1.05	18.77	1.51	17.72	3.09	14.74	1.16	17.07	2.28
2,6-Dimethoxyphenol	8.06	0.49	7.69	0.24	7.98	1.10	7.39	0.00	7.66	0.35
Furfural	1.48	0.15	2.03	0.15	1.42	0.10	1.19	0.13	1.30	0.09

Table 5
Concentration and standard deviation ($\mu\text{g/L}$) of volatile compounds of Area 1 Corvino wines.

	Yeast 1		Yeast 2		Yeast 3		Yeast 4		Spontaneous	
	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
Alcohols										
1-Butanol	104.36	13.96	110.33	1.24	91.85	17.30	67.82	2.03	103.39	11.81
2-Butanol	4080.67	103.33	4778.01	731.57	6011.68	215.35	2716.06	55.06	6474.19	1004.08
1-Pentanol	73.85	1.26	81.61	1.87	72.12	0.37	80.06	0.77	76.03	2.92
Isoamyl alcohol	156916.69	8140.96	176681.52	2115.33	186962.14	1415.80	120782.20	1694.02	160622.13	8400.54
Phenethyl alcohol	13170.04	727.63	14139.50	813.17	13595.22	806.34	10471.27	1232.96	12191.86	153.48
Methionol	573.99	65.60	263.87	19.79	499.98	4.02	568.75	49.86	271.58	10.57
C₆ alcohols										
1-Hexanol	2313.95	89.31	2519.78	58.34	2127.75	14.91	2422.70	2.93	2648.03	151.55
trans-3-Hexen-1-ol	30.09	1.75	36.47	2.02	44.10	2.18	38.70	0.41	23.22	0.59
cis-3-Hexen-1-ol	45.37	7.21	69.46	2.66	61.30	5.48	59.27	7.49	63.00	4.60
cis-2-Hexen-1-ol	13.04	0.28	12.94	0.57	13.42	0.42	13.64	0.32	12.99	0.73
Acetate esters										
Isoamyl acetate	445.65	76.40	561.02	55.76	416.62	6.12	443.38	3.16	485.55	81.76
n-Hexyl acetate	5.78	0.78	8.08	0.09	4.97	0.69	9.97	0.20	7.68	1.39
2-Phenethyl acetate	22.81	0.87	20.25	1.34	19.44	0.79	19.00	1.82	25.49	2.40
Ethyl acetate	32724.56	501.48	43430.90	15312.31	40203.87	46.88	32663.26	6468.05	72443.24	20639.62
Branched-chain fatty acids ethyl esters										
Ethyl 2-methyl butanoate	2.18	0.07	2.96	0.13	3.67	0.21	1.78	0.04	2.32	0.16
Ethyl 3-methyl butanoate	1.77	0.28	2.24	0.34	4.88	0.67	1.54	0.06	2.14	0.16
Ethyl fatty acids esters										
Ethyl butanoate	165.63	21.60	247.91	23.29	168.97	11.94	163.61	47.36	135.33	11.25
Ethyl hexanoate	520.90	138.35	797.21	105.09	569.31	1.63	601.95	46.45	429.34	22.54
Ethyl octanoate	282.00	100.43	402.37	141.67	344.99	9.84	306.05	10.85	231.49	12.04
Ethyl decanoate	68.15	21.82	100.22	7.11	70.07	10.47	75.36	4.00	45.03	1.01
Other esters										
Ethyl 3-hydroxybutanoate	239.60	32.98	288.18	14.26	371.56	2.71	131.22	0.09	252.11	18.07
Ethyl 2-hydroxyhexanoate	0.37	0.02	0.89	0.06	1.24	0.08	0.42	0.03	1.07	0.10
Fatty acids										
3-Methylbutanoic acid	391.60	4.19	404.75	12.46	494.08	53.73	350.12	0.08	305.08	18.17
Hexanoic acid	3647.93	615.24	6145.21	53.94	4300.07	157.32	4148.32	227.00	3271.93	170.77
Octanoic acid	5932.80	606.00	7152.51	415.59	6203.58	46.87	6116.99	296.59	5045.65	36.26
Terpenoids										
cis-Linalooloxide	0.24	0.02	0.12	0.01	0.21	0.01	0.10	0.00	0.26	0.02
trans-Linalooloxide	0.44	0.04	0.64	0.08	0.30	0.03	0.40	0.04	0.43	0.01
Linalool	3.86	0.04	6.02	0.11	4.51	0.01	4.65	0.22	4.73	0.40
Geraniol	5.29	0.30	4.90	0.69	8.24	0.97	11.53	0.89	3.32	0.04
β -Citronellol	10.82	0.88	12.75	1.48	10.44	0.78	7.21	0.88	11.15	0.05
α -Terpineol	0.76	0.10	1.34	0.09	0.96	0.10	0.75	0.06	1.03	0.12
3-Carene	0.05	0.00	0.09	0.01	0.07	0.01	0.05	0.00	0.06	0.01
α -Phellandrene	1.43	0.03	1.77	0.05	1.81	0.16	3.19	0.52	1.37	0.01
α -Terpinen	0.02	0.00	0.03	0.00	0.03	0.00	0.04	0.00	0.04	0.01
β -Myrcene	1.86	0.33	2.35	0.14	2.34	0.21	4.14	0.68	1.78	0.01
Limonene	0.18	0.02	0.19	0.01	0.23	0.01	0.30	0.01	0.20	0.00
1,8-Cineol	0.07	0.01	0.10	0.01	0.07	0.00	0.08	0.01	0.06	0.01
p-Cymene	0.11	0.01	0.17	0.03	0.12	0.01	0.13	0.01	0.11	0.01
Terpinolene	0.10	0.01	0.10	0.01	0.12	0.01	0.16	0.01	0.13	0.01
Terpinen-1-ol	0.29	0.02	0.31	0.01	0.27	0.00	0.32	0.01	0.28	0.01
Terpinen-4-ol	0.02	0.00	0.00	0.00	0.02	0.00	0.04	0.01	0.03	0.00
Nerol	0.72	0.04	0.61	0.04	0.68	0.13	0.59	0.02	0.67	0.11
Norisoprenoids										
β -Damascenone	1.60	0.15	1.80	0.08	1.47	0.10	2.14	0.25	1.78	0.14
3-Oxo- α -ionol	1.16	0.01	1.21	0.03	1.20	0.11	1.40	0.06	1.27	0.05
3-Hydroxy- β -damascone	0.09	0.01	0.07	0.00	0.12	0.01	0.09	0.00	0.08	0.01
Vitispirane	0.33	0.04	0.15	0.01	0.50	0.01	0.60	0.01	0.49	0.02
TPB	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00
TDN	0.07	0.00	0.06	0.01	0.09	0.01	0.10	0.01	0.09	0.00
Benzenoids and others										
Benzyl Alcohol	26.95	1.43	42.36	1.23	43.91	1.76	36.33	0.45	52.63	4.27
Vanillin	4.85	0.69	4.70	0.49	5.08	0.70	5.45	0.78	4.42	0.41
Vanillyl alcohol	9.45	0.96	14.17	3.36	12.24	2.44	8.83	0.13	13.43	2.39
Ethyl vanillate	156.19	1.63	148.70	5.85	140.02	7.86	99.91	5.70	148.98	1.68
Methyl vanillate	14.92	0.42	14.91	0.44	14.31	0.23	16.01	1.86	14.71	0.12
Benzaldehyde	14.69	0.42	14.54	0.32	14.62	0.04	14.57	0.01	14.46	0.08
Eugenol	0.71	0.12	0.67	0.10	0.76	0.16	0.73	0.03	0.51	0.03
Methyl salicylate	1.63	0.02	1.70	0.01	2.36	0.01	2.04	0.20	2.11	0.07
γ -Decalactone	3.28	0.49	2.93	0.25	2.70	0.15	2.79	0.09	3.02	0.21
δ -Decalactone	13.22	0.75	18.56	1.99	13.81	1.97	14.77	1.90	14.43	1.03
2,6-Dimethoxyphenol	7.80	0.14	10.29	0.49	10.30	0.25	9.00	1.01	10.52	0.23
Furfural	1.49	0.26	2.26	0.40	1.97	0.06	1.78	0.25	1.45	0.11

Table 6
Concentration and standard deviation ($\mu\text{g/L}$) of volatile compounds of Area 2 Corvino wines.

	Yeast 1		Yeast 2		Yeast 3		Yeast 4		Spontaneous	
	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
Alcohols										
1-Butanol	131.94	16.38	114.40	1.87	102.35	4.77	73.87	7.19	96.20	4.69
2-Butanol	4951.13	861.54	5368.33	169.36	7029.57	188.93	3672.23	74.94	7167.72	1027.55
1-Pentanol	77.66	4.46	82.93	3.33	67.65	4.47	66.80	3.99	75.77	1.82
Isoamyl alcohol	176112.82	1532.93	167756.99	147.62	194558.32	1373.71	143779.22	5164.50	158640.69	1414.31
Phenethyl Alcohol	13706.06	1738.29	10643.55	524.84	12352.42	1209.08	12007.38	178.93	11476.68	193.37
Methionol	531.41	69.40	351.46	40.55	552.42	66.48	647.60	16.45	329.99	36.18
C₆ alcohols										
1-Hexanol	3889.71	404.39	4118.86	414.94	3187.19	33.55	2978.89	265.87	4301.85	263.72
<i>trans</i> -3-Hexen-1-ol	57.67	8.17	69.06	4.21	53.13	0.06	43.78	6.38	29.47	1.41
<i>cis</i> -3-Hexen-1-ol	52.40	8.35	65.24	7.35	50.98	8.31	41.68	6.29	41.90	3.36
<i>cis</i> -2-Hexen-1-ol	13.00	1.35	13.57	0.37	12.56	0.29	14.40	0.18	13.04	0.51
Acetate esters										
Isoamyl acetate	438.79	52.55	828.31	64.58	749.99	16.74	389.63	44.82	597.35	121.17
n-Hexyl acetate	10.70	0.59	21.81	0.68	12.59	1.23	12.58	1.23	17.88	2.07
2-Phenethyl acetate	24.86	2.03	26.64	1.53	26.42	1.27	21.71	0.88	35.52	7.75
Ethyl acetate	30479.75	3829.23	37405.76	2927.84	30276.97	6317.38	22150.79	9514.40	90134.73	13811.16
Branched-chain fatty acids ethyl esters										
Ethyl 2-methyl butanoate	3.21	0.40	3.15	0.19	3.79	0.28	2.37	0.21	2.25	0.01
Ethyl 3-methyl butanoate	2.28	0.35	3.89	0.23	5.59	0.74	3.14	0.21	1.64	0.21
Ethyl fatty acids esters										
Ethyl butanoate	142.77	6.11	207.93	29.42	120.71	15.65	140.23	11.20	137.76	3.92
Ethyl hexanoate	511.27	38.58	659.21	23.19	477.27	40.21	492.09	21.96	491.09	19.09
Ethyl octanoate	266.49	35.65	311.05	2.70	259.87	16.40	232.56	4.03	213.64	52.79
Ethyl decanoate	55.05	1.06	63.34	0.28	61.30	3.95	59.40	2.58	38.96	12.01
Other esters										
Ethyl 3-hydroxybutanoate	287.77	1.39	302.23	1.71	366.64	38.80	133.24	0.77	250.95	32.86
Ethyl 2-hydroxyhexanoate	0.56	0.08	0.89	0.04	1.46	0.08	0.42	0.05	0.77	0.07
Fatty acids										
3-Methylbutanoic acid	433.98	92.23	392.91	19.57	582.06	19.77	412.72	57.09	261.50	21.67
Hexanoic acid	3345.15	43.58	4422.53	394.77	2982.90	280.79	3088.65	88.44	3394.91	205.14
Octanoic acid	5453.82	241.59	5751.54	28.21	4989.91	320.95	5072.26	77.56	5107.78	453.48
Terpenoids										
<i>cis</i> -Linalooloxide	0.58	0.04	0.34	0.02	0.45	0.01	0.51	0.06	0.38	0.06
<i>trans</i> -Linalooloxide	0.60	0.08	0.42	0.02	0.52	0.02	0.49	0.03	0.52	0.01
Linalool	7.43	1.05	8.92	0.09	6.34	0.56	6.15	0.64	7.28	0.04
Geraniol	3.82	0.43	3.12	0.12	5.21	0.11	10.02	1.65	4.02	0.16
β -Citronellol	9.23	1.37	10.07	1.46	8.71	0.70	6.54	1.11	8.31	0.58
α -Terpineol	1.84	0.28	2.19	0.22	1.43	0.12	1.36	0.20	1.78	0.09
3-Carene	0.05	0.01	0.05	0.00	0.05	0.01	0.05	0.01	0.05	0.00
α -Phellandrene	2.73	0.15	2.28	0.31	2.18	0.40	1.81	0.14	1.31	0.06
α -Terpinen	0.16	0.05	0.07	0.01	0.14	0.02	0.08	0.01	0.06	0.00
β -Myrcene	3.43	0.34	3.01	0.13	2.83	0.29	2.39	0.13	1.70	0.08
Limonene	0.87	0.08	0.73	0.11	0.81	0.15	0.60	0.01	0.54	0.02
1,8-Cineol	0.12	0.01	0.12	0.02	0.13	0.02	0.10	0.01	0.12	0.03
<i>p</i> -Cymene	0.30	0.04	0.20	0.04	0.27	0.04	0.21	0.01	0.15	0.01
Terpinolene	0.28	0.01	0.28	0.00	0.23	0.04	0.25	0.05	0.20	0.01
Terpinen-1-ol	0.29	0.03	0.23	0.04	0.21	0.04	0.31	0.02	0.12	0.16
Terpinen-4-ol	0.32	0.00	0.84	0.06	5.31	1.05	3.34	0.19	2.01	0.01
Nerol	0.02	0.00	0.02	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Norisoprenoids										
β -Damascenone	2.60	0.09	2.14	0.28	2.01	0.01	1.70	0.15	2.62	0.20
3-Oxo- α -ionol	0.89	0.03	0.83	0.04	0.91	0.04	0.93	0.05	0.89	0.08
3-Hydroxy- β -damascone	0.13	0.01	0.08	0.00	0.10	0.01	0.09	0.01	0.07	0.00
Vitispirane	8.54	0.95	9.93	9.05	11.05	1.24	11.44	2.31	8.77	1.46
TPB	0.07	0.01	0.09	0.08	0.10	0.00	0.11	0.01	0.08	0.02
TDN	1.71	0.20	3.31	0.30	1.65	0.33	1.83	0.30	2.06	0.13
Benzenoids and others										
Benzyl Alcohol	17.32	0.07	10.03	0.85	12.72	1.32	15.85	0.06	14.66	0.04
Vanillin	4.82	0.56	3.88	0.11	5.28	0.22	5.37	0.62	4.25	0.73
Vanillyl alcohol	9.39	0.16	8.97	0.91	7.96	2.25	6.32	0.93	8.25	1.09
Ethyl vanillate	151.67	11.58	162.14	9.35	140.75	2.08	145.67	7.66	153.17	4.89
Methyl vanillate	16.41	0.39	17.48	0.78	14.70	0.64	14.27	0.15	16.62	0.15
Benzaldehyde	14.44	0.22	14.31	0.08	14.34	0.10	14.46	0.01	14.37	0.05
Eugenol	1.11	0.37	1.08	0.25	1.60	0.35	1.04	0.04	0.52	0.02
Methyl salicylate	0.76	0.00	0.68	0.04	0.84	0.11	0.96	0.01	0.24	0.01
γ -Decalactone	4.27	0.48	3.94	0.08	2.72	0.36	3.10	0.01	3.81	0.41
δ -Decalactone	14.36	0.84	17.00	2.15	14.71	0.18	13.64	1.60	14.47	0.66
2,6-Dimethoxyphenol	10.16	0.64	9.67	0.06	10.05	0.07	10.45	1.12	10.62	0.81
Furfural	1.45	0.06	1.63	0.06	1.52	0.16	1.38	0.04	1.27	0.13

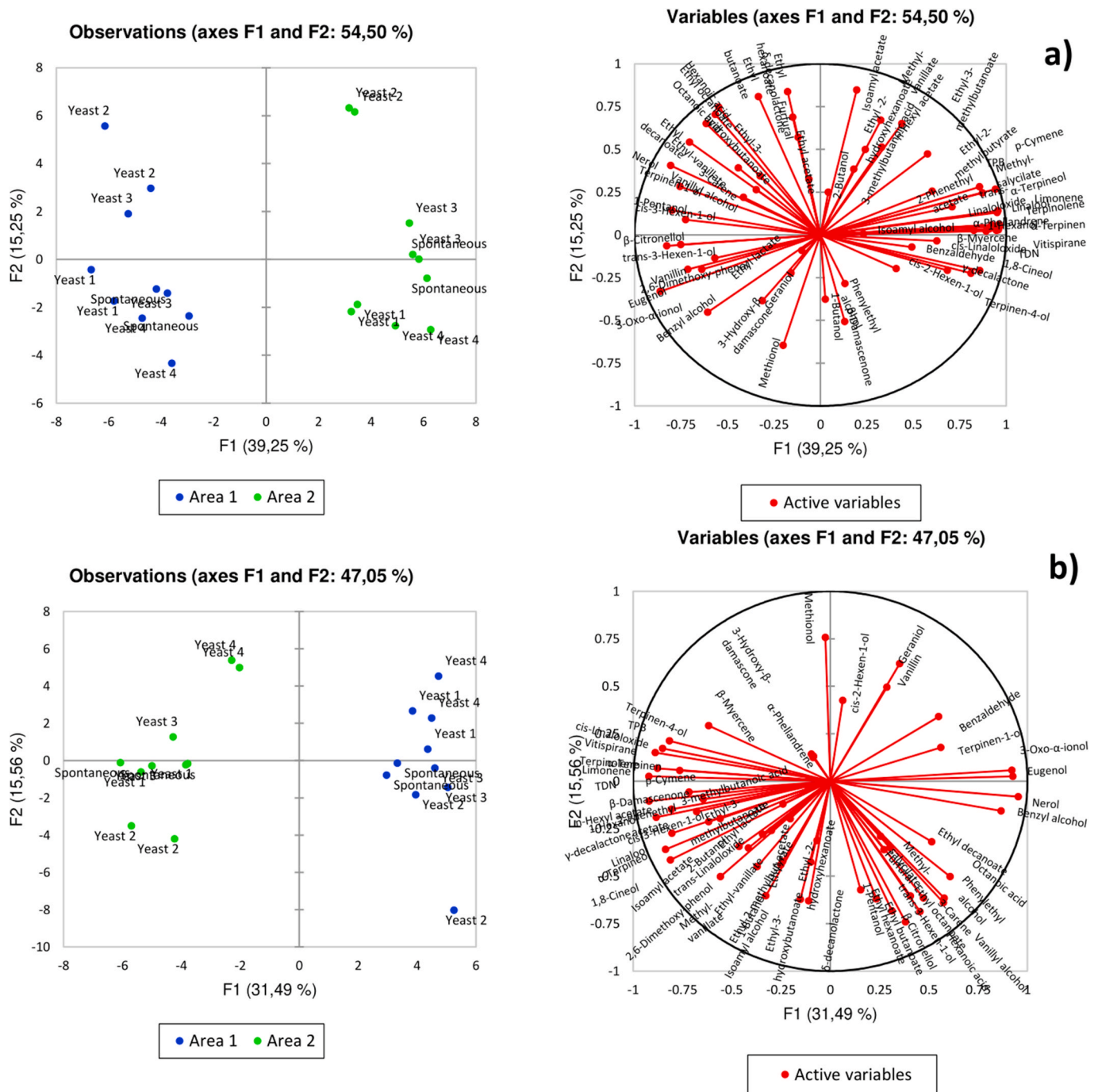


Fig. 1. Principal Component Analysis of volatile compounds in (a) Corvina and (b) Corvinone wines.

of Corvina wines (Fig. 1a), 54.5% of the total variance was explained with the first two principal components. PC1, accounting for 39.25% of the variance, was associated with grape origin, while PC2, accounting for 15.25% of the total variance, differentiated yeasts/inoculation strategy. In the case of Corvinone wines (Figs. 1b), 47.05% of the total variance was explained with the first two principal components, with PC1 accounting for 31.49% of the total variance and being associated with grape origin, and PC2 accounting for 15.55% of the total variance, and mostly differentiating according to the yeast strain/inoculation. Accordingly, grape origin was associated with greater variation in wine volatile composition compared to yeast strain/inoculation. This observation is in agreement with Robinson et al. (2011), and highlights the importance of grape batch/vineyard site selection in the production of

wines with specific aroma characteristics.

Terpenes were among the volatiles mostly associated with area-driven differences. The importance of grape variety to terpenes content is well known (Mateo & Jimenez, 2000), and within the same variety terpenes profile can differ due to vineyard location (Sabon, De Revel, Kotseridis, & Bertrand, 2002; Robinson et al., 2011; Antalic, Šuklje, et al., 2015; Celik et al. 2015; Wen et al., 2015; Slaghenaufl et al., 2019) soil composition (Jackson, 2008), sunlight exposure (Bureau, Baumes, & Razungles, 2000) and canopy management (Zoeklein, Wolf, Pélanne, Miller, & Birkenmaier, 2008). In the case of Corvina, the average total terpenes content of wines from Area 2 was 29% higher than wines from Area 1, whereas Area 2 Corvinone wines showed average terpenes content 10% higher than Area 1. Although terpenes

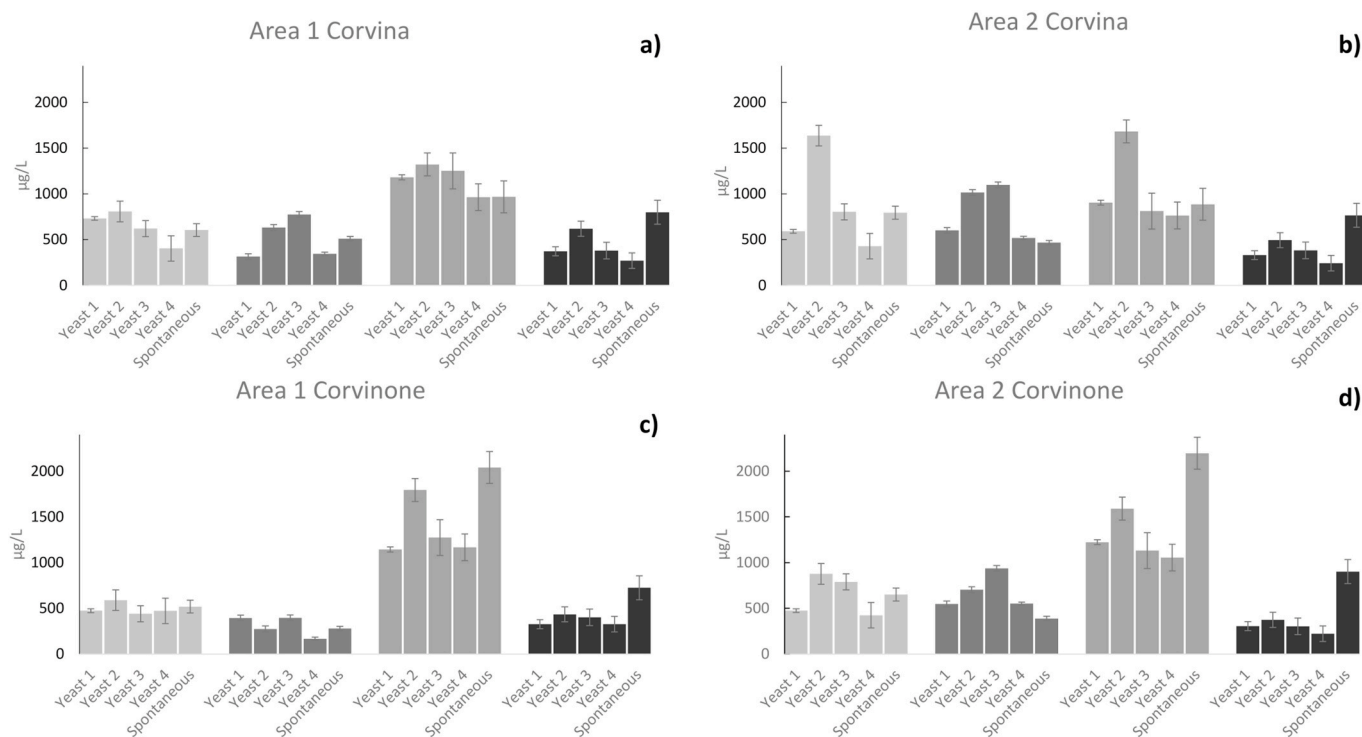


Fig. 2. Total acetate esters, total branched-chain fatty acid ethyl ester (multiplied by 100), total ethyl fatty acids esters and ethyl acetate content (divided by 100) in (a) Area 1 Corvina wines, (b) Area 2 Corvina wines, (c) Area 1 Corvinone wines and (d) Area 2 Corvinone wines.

influence on wine aroma is mostly associated with white wines, a number of recent reports have highlighted their importance also in red wine aroma (Antalick, Tempère, et al., 2015; Slaghenaufl & Ugliano, 2018). Norisoprenoids discriminated wines based on grape origin, again with wines from Area 2 showing higher concentrations. This was mostly due to the higher content of vitispirane and TDN, in particular in Corvinone wines, where TDN approached and in some cases exceeded the detection threshold of 2 $\mu\text{g/L}$ reported by Sacks et al. (2012). As for the other potent aroma compound β -damascenone, it did not show significant difference according to area alone, although a significant degree of area*yeast was observed. These data confirm prior observation concerning the impact of grapegrowing conditions (Gerdes, Winterhalter, & Ebeler, 2001; Lloyd et al., 2011; Sefton, Skouroumounis, Elsey, & Taylor, 2011), and yeast strain (Lloyd et al., 2011) on wine norisoprenoids. Finally, within the same variety, benzenoids, such as benzyl alcohol, vanillin, eugenol, 2,6-dimethoxyphenol, and C_6 alcohols, were also influenced grape origin, although no clear pattern could be observed.

Compared to grapes area of origin, yeast strain was found to affect wine volatile composition to a lower extent. This can be clearly seen in Fig. 1a and b, where discrimination of wines based on yeast strain was associated with PC2, accounting for less than 16% of the total variance. Esters, alcohols and fatty acids were most frequently associated with statistically-significant variations due to yeast strain alone, in agreement with their strong relationship with fermentation metabolism (Ugliano & Henschke, 2009). However, several aroma compounds present in concentrations exceeding their respective odor thresholds were largely affected by yeast, and some strain-related patterns were also observed. This was especially the case for esters, potent aroma compounds associated with wine fruitiness, for which yeast 2 (AWRI 796) produced in all wines higher concentrations of isoamyl acetate, hexyl acetate, and ethyl fatty acid esters. The fact that wine esters content was influenced by yeast strain was somewhat expected, as several authors have reported a major influence of yeast strain on wine esters profile (Ugliano & Henschke, 2009) related to different levels of expression of acetyl transferases for acetate esters (Lilly, Lambrechts, & Pretorius, 2000) and

esterification of activated fatty acids (acyl-CoA) during lipid biosynthesis for ethyl esters (Suomalainen, 1981). In most of the published reports, the relationship between esters and yeast strain was studied by inoculating a single must with different yeasts. However, when different combinations of yeast and musts are studied, as in the case of the present work, the situation appears more complex. The data in Fig. 2 indicate indeed that the magnitude of yeast effect can vary significantly depending on grape composition or variety. In fact if we consider the data of wine sets from individual grape batches it can be seen that yeast-induced variations in acetate esters concentrations were up to five folds in the case of Corvina 2, whereas they were around two folds in other cases. At the same time, within the same variety, variations due to area of grape origin were generally of a factor two, so that for acetate esters yeast*area was more impactful than yeast alone. Altogether these observations indicate that the extent to which yeast strain can impact ester formation during fermentation is strictly modulated by grape composition, so that in certain fermentations the effect of yeast can be relatively small, in particular for acetate esters. Factors accounting for such different behaviors are complex, (Antalick, Perello, & de Revel, 2014), and the importance of YAN and lipid composition of the grapes has been recently highlighted (Antalick, Šuklje, et al., 2015). However, in the present study, no correlation was found between must YAN and wine ester content, possibly due to a concomitant influence of other grape compositional factors (Keyzers & Boss, 2010). In any case, considering ester values across the entire experiment, yeast 2 was characterized by increased production of total ethyl esters and isoamyl acetate.

Yeast-driven patterns were also observed for certain alcohols, for example for the *Saccharomyces cerevisiae* x *Saccharomyces kudriavzevii* hybrid (yeast 1), higher levels of 1-butanol but not of 2-butanol were always observed, potentially reflecting specific metabolic traits for butanol isomers pathways (Generoso, Schadoweg, Oreb, & Boles, 2015) as well as possible differences in nitrogen utilization patterns in *S. kudriavzevii* compared to *S. cerevisiae* (Štrbny, Gamero, Pérez-Torradó, & Querol, 2015). Certain compounds primarily originating in grape

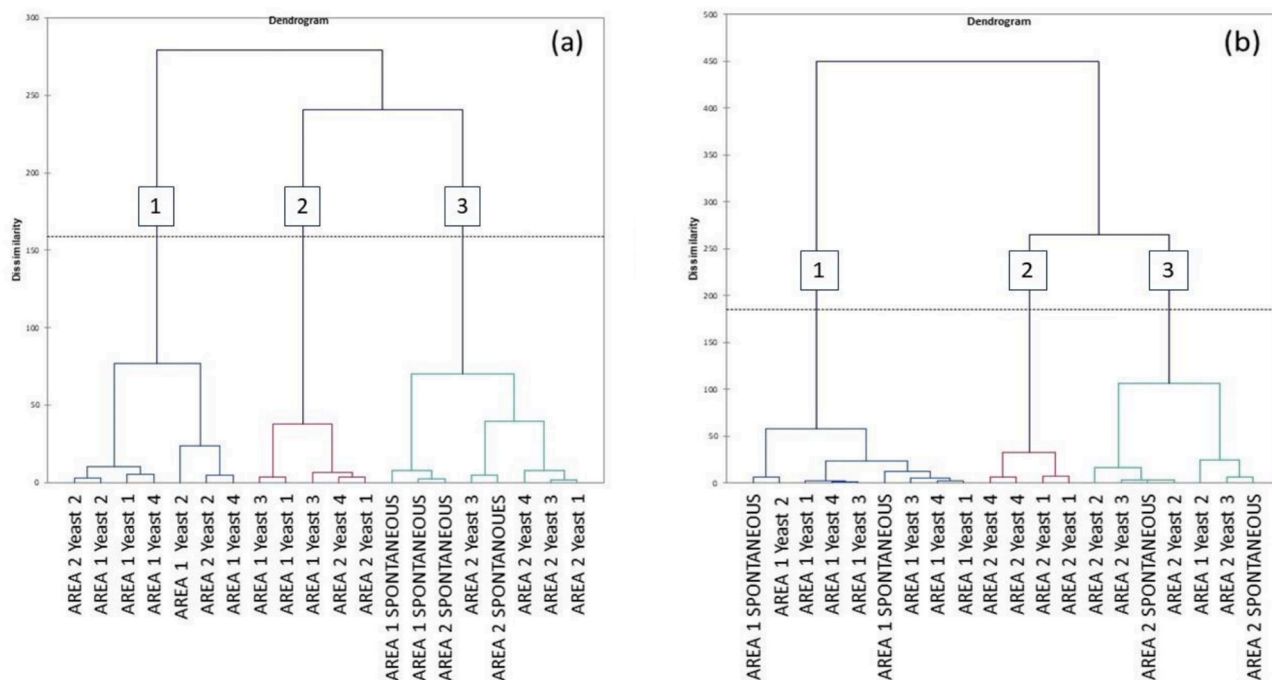


Fig. 3. Hierarchical Cluster Analysis of (a) Corvina and (b) Corvinone sorting tasks data.

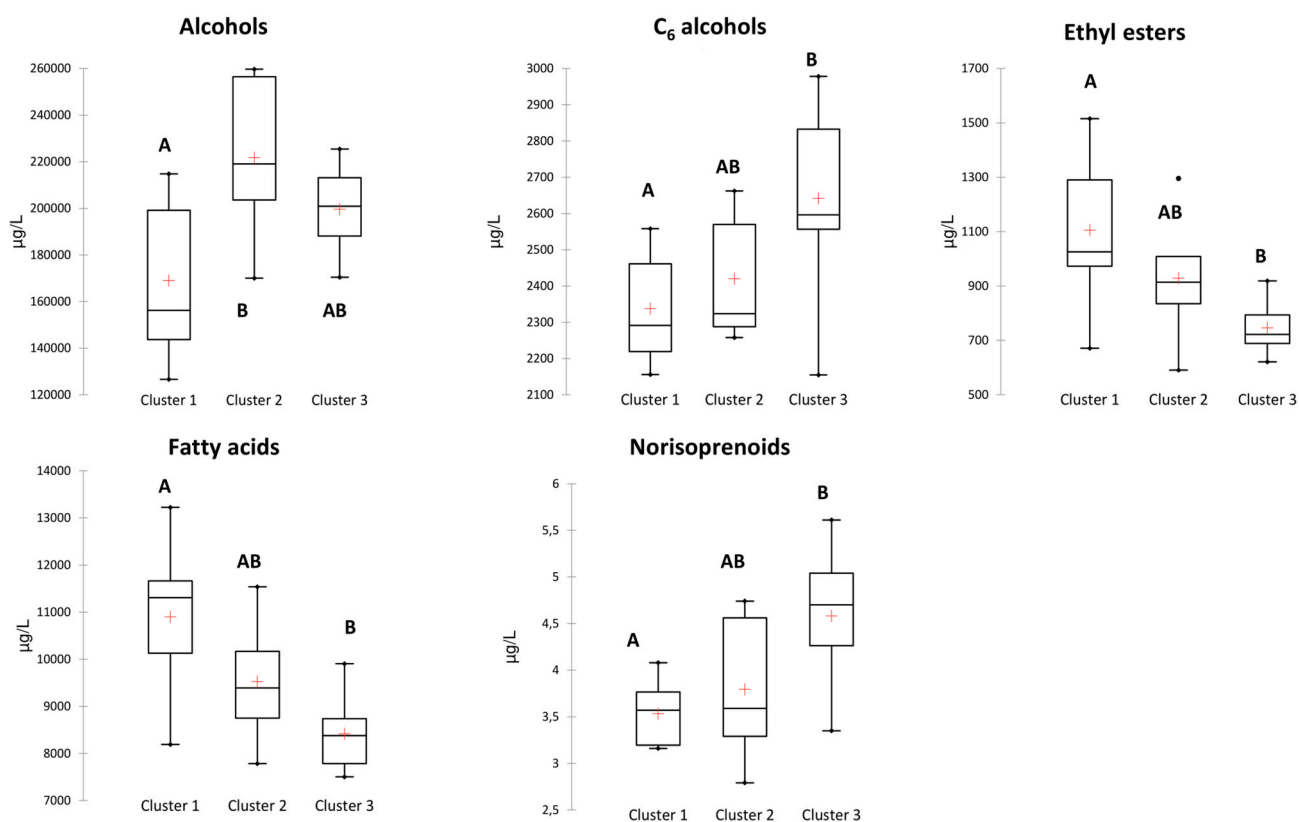


Fig. 4. Concentration ranges of chemical classes with significant differences (capital letters) according to Mann-Whitney test ($\alpha = 0.05$) of Corvina clusters obtained from Hierarchical Cluster Analysis. Alcohols include 1-butanol, 2-butanol, 1-pentanol, isoamyl alcohol, phenethyl alcohol and methionol; C_6 alcohols include 1-hexanol, *trans*-3-hexen-1-ol, *cis*-3-hexen-1-ol and *cis*-2-hexen-1-ol; Ethyl esters include ethyl butanoate, ethyl hexanoate, ethyl octanoate and ethyl decanoate; Fatty acids include 3-methylbutanoic acid, hexanoic acid and octanoic acid; Norisoprenoids include 3-oxo- α -ionol, β -damascenone, 3-hydroxy- β -damascone, vitispirane, TPB, TDN.

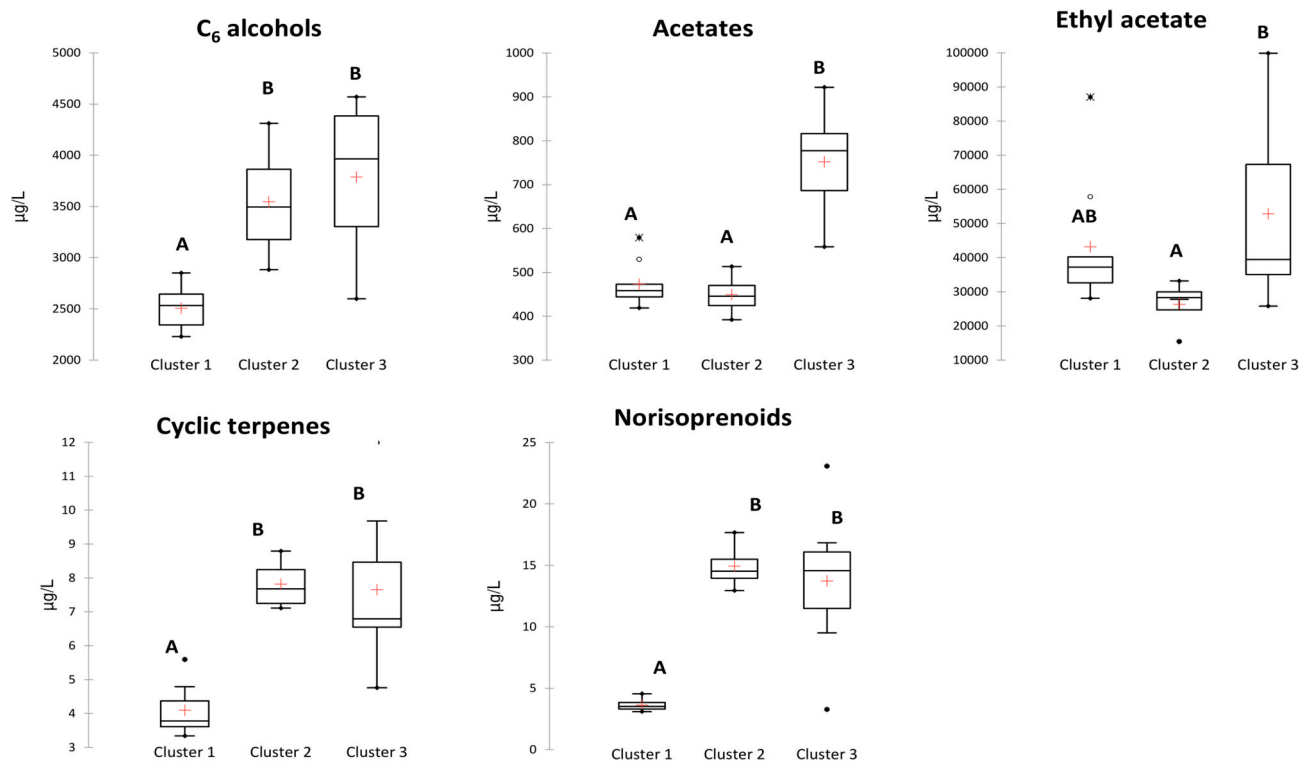


Fig. 5. Concentration ranges of chemical classes with significant differences (capital letters) according to Mann-Whitney test ($\alpha = 0.05$) of Corvino clusters obtained from Hierarchical Cluster Analysis. C₆ alcohols include 1-hexanol, *trans*-3-hexen-1-ol, *cis*-3-hexen-1-ol and *cis*-2-hexen-1-ol; Acetate esters include isoamyl acetate, 2-phenethyl acetate and n-hexyl acetate; Cyclic terpenes include *cis*- and *trans*-linalooloxide, α -terpineol, 3-carene, α -phellandrene, α -terpinen, limonene, 1,8-cineole, *p*-cymene, terpinolene, terpinen-1-ol, terpinen-4-ol; Linear terpenes include linalool, geraniol, β -citronellol, β -myrcene and nerol; Norisoprenoids include 3-oxo- α -ionol, β -damascenone, 3-hydroxy- β -damascone, vitispirane, TPB, TDN.

were also affected by yeast strain. Among these, geraniol, 3-hydroxy- β -damascone, and vanillin were for the most part affected by yeast and not by area. *S. cerevisiae* is able to synthesize terpenes such as geraniol, but the contribution of this pathway is expected to be minor compared to release from glycosidic precursors and/or bio transformation of other terpenes (Ugliano, Bartowsky, McCarthy, Moio, & Henschke, 2006). A compound typically associated with use of oak but observed in the present study was ethyl vanillate, which was the most abundant benzenoid found in Corvina and Corvino. Vanillin derivatives can be produced by yeast as by-products of ferulic acid metabolism (Huang, Dostal, & Rosazza, 1993) and some of these could be esterified by yeast or through acid catalysed reactions. An association between fermentation and increase in ethyl vanillate concentration has been recently reported in Corvina (Slaghenaufi et al., 2019), which could explain the observation of a significant influence of yeast.

With regards to spontaneous fermentation, for each grape batch this resulted in lower content of *trans*-3-hexenol and 3-methylbutanoic acid, whereas ethyl acetate was more abundant in all spontaneous fermentations compared with the average concentrations obtained in all from inoculated fermentations (Supplementary 5). Other compounds were only occasionally affected depending on grape batch.

3.3. Sensory characteristics of wines in relationship to volatile composition

In order to evaluate the existence of sensorially relevant patterns that could be attributed to one or more of the studied variables, a sorting task was carried out. This approach has been previously used to differentiate wines based on sorting samples into different groups according to sensory similarities (Alegre et al., 2017). The results of the sorting task are shown in Fig. 3, indicating that, for each variety, panellists were able to group samples in different clusters exhibiting odor similarities. In

general, biological replicates were projected in the same groups, suggesting that they were similarly perceived by panellists. In the case of Corvina (Fig. 3a), a larger cluster consisted of seven wines, five of which were obtained from Area 1 grapes. A second cluster consisted of five wines all associated with both areas, and finally a third cluster contained eight wines, six of which were associated with Area 2. Interestingly, all spontaneous fermentations were sorted in the third group and all Yeast 2 samples were in cluster 1, regardless of grape origin. Also in the case of Corvino (Fig. 3b) three clusters were obtained, with the first largest group consisting of ten wines, nine of which were associated with Area 1. The second cluster consisted of four wines, all from Area 2, fermented with yeast 2 and 4. Finally, a third cluster consisting of six wines was obtained, five of which were associated with Area 2. Samples obtained by means of spontaneous fermentations were separated depending on area.

Altogether, these results indicate that, within each variety, odor similarities perceived across samples were primarily associated with area, which in turn indicate a primary role of grape compositional features. This was particularly clear in the case of Corvino, where area of grape origin was clearly the main element of distinction, with yeasts 2 and 3 exhibiting further elements of odor differentiation within Area 2. Analysis of classes of compounds discriminating clusters to a statistically significant degree was carried out by Mann-Whitney test (Supplementary 6), with compounds belonging to the same chemical class or, where possible, sharing similar odor properties grouped in the same class. In the case of Corvino (Fig. 4), cyclic terpenes, C₆ alcohols, norisoprenoids, and acetate esters were likely to contribute to these odor differences. Considering the concentrations detected and the odor thresholds of the compounds included in each group, the difference in norisoprenoids are likely to contribute to the perceived differences, as well as those in esters. As for cyclic terpenes, individual compounds in this group were in no case detected in concentrations exceeding their

reported thresholds (Antalick, Šuklje, et al., 2015) but mutual synergistic effects have been reported for these compounds (Niu, Sun, Xiao, Wang, & Wang, 2018), so their sensory contribution is also possible.

In the case of Corvina sorting of the samples based on odor similarity was less clearly attributable to either area or yeast, although the influence of area was still clear while a certain influence of yeast strain could be observed. Yeast 2 samples were indeed all grouped in one cluster and all samples from spontaneous fermentation were grouped in another cluster, regardless of area. Among compounds discriminating clusters to a statistically significant degree (Supplementary 6), ethyl esters differentiated cluster 1 from the other two, together with fatty acids (Fig. 5). The combined influence of these two classes of compounds could be key to the odor similarities existing across cluster 1, as they act synergistically towards increased expression of wine fruity attributes (San-Juan, Ferreira, Cacho, & Escudero, 2011). Interestingly, although samples from spontaneous fermentation were grouped in cluster 3, this cluster was not significantly higher in ethyl acetate, which we observed to be a metabolic marker of spontaneous fermentations. Perceptual interactions have been described for ethyl acetate in combination with various other esters including ethyl hexanoate (Niu, Yao, et al., 2018), which was the main component of the ethyl esters group of Fig. 5. Accordingly, the odor contribution of ethyl acetate could be modulated by ethyl esters concentrations.

4. Conclusion

In conclusion, this study investigated the contribution of different variables of oenological relevance on volatile composition and aroma similarities of Corvina and Corvinone wines. The results showed that most volatile compounds that are thought to influence wine aroma such as terpenes, norisoprenoids, and to a certain extent esters deriving from yeast metabolism, were primarily affected by grape composition.

Yeast alone can affect specific classes of volatiles, among which esters are the most sensorially relevant, and one strain was characterized by increased ester production in all fermentations. Sensory analysis also indicated that grape origin could be more often associated with clusters of wines having similar odor properties. However, spontaneous fermentations were characterized by high concentration of ethyl acetate regardless of grape variety and area of origin, and this was also reflected in the distinctive aroma character of the wines. Likewise, the high levels of fruity esters obtained with one strain resulted in wines with distinctive aromas.

No specific odorant responsible for such differences could be detected, although considering that Corvina and Corvinone are non-aromatic grape varieties, this had to be somewhat expected. Our data however indicate that differences due to grape composition (and therefore potentially to grape origin) and, to a lesser extent, to yeast and yeast*–composition interaction, do exist and can be perceived upon tasting. These are likely to be due to the combined contribution of different classes of odorants, showing either synergistic or antagonistic effects. Classes of volatile compounds that appears to be mostly associated with odor similarity patterns in Corvina and Corvinone wines included various esters, norisoprenoids, and cyclic terpenes, paving the way for further investigation concerning the chemical bases of the aroma distinctiveness of these two wines as well as of other red wines.

CRedit authorship contribution statement

Giovanni Luzzini: Formal analysis, Investigation, Visualization, Writing - original draft. **Davide Slaghenaufl:** Resources, Review, Supervision. **Francesca Pasetto:** Investigation. **Maurizio Ugliano:** Conceptualization, Methodology, Resources, Writing - review & editing.

Declaration of competing interest

None.

Acknowledgements

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lwt.2021.111120>.

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