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

DOI: https://doi.org/10.20870/oeno-one.2022.56.4.5520

OPEN ACCESS

ORIGINAL RESEARCH ARTICLE

Aroma and terpenoid profile of Chardonnay clone 809: from berry to sparkling wine

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Associate editor:
Maurizio Ugliano

Received:
24 April 2022

Accepted:
4 October 2022

Published:
10 November 2022

ABSTRACT

Clone selection is a viticultural practice applied to evaluate the behaviour and agronomic and flavour characteristics of a material in a determined environment to obtain wines with different aromatic attributes. The clones of the cultivar Chardonnay comprise aromatic and non-aromatic clones that can provide different sensorial qualities. This work aimed to evaluate the volatile compounds formed in clone 809 (aromatic) to produce a more aromatic sparkling wine in Serra da Mantiqueira (southeast Brazil) and compare it to clone 76 (non-aromatic) from the berry to the wine. The vineyards are in Caldas (Minas Gerais – Brazil) at the Agronomical Research Centre of Minas Gerais (EPAMIG), and the vinification was performed using the Champenoise method (18 months in *sur lie*). HS-SPME/GC-MS identified free volatile compounds in two consecutive seasons, 2017 and 2018, in grape, musts, base wines and sparkling wines. The number and abundance of monoterpenoid compounds in clone 809 were statistically significant compared to clone 76 for all sample steps. Multivariate and principal component analyses (PCA) made it possible to differentiate the base and sparkling wines by the clones in both vintages for the second component (16.8 %). Furthermore, the sparkling wines of clone 809 were discriminated for the third component (15.1 %) by the monoterpenes: α-terpineol, linalool, ß-myrcene, hotrienol, nerol oxide, and limonene. The data suggest that Chardonnay clone 809 can contribute to obtaining a sparkling wine with an additional floral and sweet character.

KEYWORDS: *Vitis vinifera*, aromatic clone, monoterpenoids, PCA analysis, HS-SPME, GC-MS

INTRODUCTION

Wine composition reflects the applied environmental, viticultural and oenological practices. Thus, wines from diverse viticultural areas, using selected yeasts in alcoholic fermentation or even berries from different clones, can result in wines with distinct sensorial characteristics. Such variability, due to monoterpene compounds (Duchêne *et al.*, 2009), thiol precursors (Nicolini *et al*., 2019) and other classes of varietal aroma compounds, is of great interest to winemakers. Studies on vine clones aim to evaluate the adaptation, production and also search for differentiated aromatic characteristics. The viability of a clone in a given location is strongly influenced by the environment (soil, climatic conditions and vineyard management techniques) and depends on the final use of the produced grapes and regulatory circumstances (Keller, 2015). For this reason, studies related to the competition of clones in new growing areas are essential.

In recent years, clones of the aromatic cultivars Gewürztraminer, Moscato Giallo and Müller Thurgau have been studied to evaluate the adaptation to specific regions and their contribution to free compounds and precursors in berries and wines (Nicolini *et al*., 2013; Nicolini *et al.*, 2016; Nicolini *et al*., 2019).

Chardonnay, a variety from Burgundy, has 31 certified clones in France (Plantgrape). Some of them are aromatic (clones 809, 1068, 1145, and 1146; Plantgrape) due to the volume of monoterpenoid compounds biosynthesised through MEP (methyl-erythritol-phosphate) and MVA (mevalonic acid) pathways. In a genetic study of the grape and wine aroma (Lin *et al*., 2019) of an aromatic clone of the Chardonnay cultivar (cv.), the authors observed an increase in monoterpenoid synthesis due to a mutant gene that expresses the enzyme 1-deoxy-D-xylose-5-synthase (DXS). Such an alteration suggests a potential catalysation for substrate build-up to produce monoterpenes through the MEP pathway.

In addition to the terpenoid pathways noted by several authors (Slaghenaufi *et al*., 2020; Yue *et al*., 2020), starting with isoprenoids units (C5) and the enzymes that contribute to the biosynthesis of these compounds in plants, Rienth *et al.* (2021) described how the biosynthesis of such aromatic compounds varies depending on berry stage. Peak production occurs during the herbaceous development phase (Phase I) of the fruit, which decreases until *véraison* (Phase II) and increases again during ripening (Phase III). Papers have described how these compounds are accumulated during the developmental stages in a cool Australian climate region in the following cultivars: Shiraz, Cabernet-Sauvignon, Riesling, Chardonnay and Pinot gris (Zhang *et al.*, 2016; Luo *et al.*, 2019). However, no data have yet been reported for Brazil´s tropical and subtropical regions.

Since Chardonnay clone 809 has more distinctive aromatic characteristics, which have already been evaluated by Duchêne *et al.* (2009) in France, this work aims to investigate the use of this material in the south of Minas Gerais (Brazil) to produce a Chardonnay sparkling wine with distinct aromatic traits and to compare the volatile compounds formed in all the process steps to Chardonnay clone 76, a non-aromatic material.

MATERIALS AND METHODS

1. Samples

1.1. Grapes

The experimental fields belong to EPAMIG (Empresa de Pesquisa Agropecuária de Minas Gerais) in Caldas and are at an elevation of 1,100 m with clay soil in the south of Minas Gerais, Brazil (21°55´S and 46°23´W). Chardonnay grapes (*Vitis vinifera* L.), clones 76 and 809, were grafted onto 1103 Paulsen (*Vitis berlandieri* x *Vitis ruprestris*) rootstock and trained on a vertical positioned trellis with 1 m between plants and 2.5 m between rows. Grapes were harvested for two consecutive vintages (2017 and 2018) in the technological maturity stage for sparkling wine production (Table S1), and the grapes for the volatile compound analysis were collected from different plants and parts of the bunch, immediately refrigerated and then stored at -80 °C before use.

1.2. Musts

Grape clusters from both clones were destemmed and pressed, adding pectolytic enzyme (Colopect VR-C at 2 g/hL from Amazon Group, Brazil) and potassium metabisulphite at 10 g/100 Kg as an antioxidant. The musts were clarified at 4 °C for 48 h. For volatile analysis, the musts were sampled after *débourbage* and stored at -80 °C.

1.3. Fermentations

After *débourbage,* the must was transferred to a 13 L Pyrex container, and *Saccharomyces bayanus* yeast and an activator (Actimax Vit 20 g/hL from Agrovin, Spain) were added for the first fermentation between 15 °C and 19 °C over 15 to 20 days. Subsequently, 50 mg/L of potassium metabisulfite was added and the base wine was clarified with bentonite (one step with 80 g/hL and a second step with 30 g/hL, from Laffort, United States) and stabilised with tartaric at -2 °C for 12 days. Concluding these steps, the base wine for volatile analysis was obtained and stored at -80 °C, and the principal amount proceeded to the second vinification stage in the bottles (*champenoise* method). The tirage liqueur, a mixture of sugars (sucrose, 26 g/L), yeast *Saccharomyces bayanus,* and coadjuvants (20 g/hL of Actimax Vit, 30 g/hL of bentonite, and 20 g/hL of Gesferm Plus from Amazon Group, Brazil), was added to each bottle for the second fermentation and ageing under lees for 18 months. The sparkling wine was stored after *dégorgement* in an underground cellar and was refrigerated before analysis. Aliquots were taken before the extraction procedure and sonicated for 2 min.

2. Volatile compound analysis

2.1. Sample preparation

Before the pool of grapes was crushed under liquid nitrogen, the skins and seeds were removed, because they are not part of the white and sparkling winemaking process. Grape powders

were stored in an ultra-freezer until sample preparation. Next, 3 g of the powder was weighed inside a 20 mL headspace screw-top vial (Agilent), which was increased to 10 g with a NaCl saturated solution (ACS grade, Sigma-Aldrich), and the vial was tightly capped with PTFE/silicone septum (Agilent) to prepare the samples of volatiles. For the musts, base wines and sparkling wines, 5 mL of the specimen was taken, and 5 mL of NaCl saturated solution was added to the vial. Before injection, as the internal standard, 50 µL of 4-methyl-2-pentanol was added at 1.6 mg/mL (Certified reference material, Sigma-Aldrich). For all samples, the analysis was performed in triplicate (analytical triplicate).

2.2. HS-SPME extraction and sampling

The extraction and concentration of the volatile compounds was performed by headspace-solid phase microextraction (HS-SPME). The samples were equilibrated for 10 min (grape berries and must) or 5 min (base and sparkling wines) in a water bath adjusted to 40 °C. Then, the SPME fibre, 50/30 µm 2-cm DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethylsiloxane) Stableflex from Supelco was inserted and equilibrated into the headspace for 50 min. Afterwards, the fibre was removed and exposed in the gas chromatography (GC) injection port at 250 °C for 5 min for desorption.

2.3. GC-MS separation and detection

The GC-MS was performed using an HP6890 (Series GC System G1530A) coupled with an HP model 5973 mass selective detector (Agilent Technologies, Palo Alto, CA) fitted with a Carbowax column (30 m x 0.25 mm x 0.25 µm). The chromatographic method for grape and must was adapted from Sun *et al.* (2011). The oven was kept at 35 °C for 5 min, ramped at 4.5 °C/min to 170 °C, then raised to 250 °C at 8 °C/min and held for 5 min. The carrier gas was helium at 1 mL/min (Analytical purity 99.999 %, White Martins, São Paulo, SP, Brazil). For the base and sparkling wines, the method was adjusted according to Carlin *et al.* (2016). The helium flow rate was 1.2 mL/min, and the ramp started at 40 °C for 5 min, was ramped at 6 °C/min to 250 °C, and then maintained for 10 min. In both methods the injector port and transfer line temperatures were kept at 250 °C.

The MS parameters were set at 70 eV, 230 °C (EI source temperature) and 150 °C (quadrupole temperature). Chemstation software was used for data acquisition, and the mass spectra were acquired over *m/z* 30 to 350.

2.4. Data process and compound assignment

The data process was performed by Agilent MassHunter Qualitative Analysis software (v. B.07.00), applying the chromatogram deconvolution tool and the compounds obtained from the National Institute of Standards and Technology library (NIST 14; score applied above 70). In addition to this evaluation, the modified Kovats retention index (RI) of these compounds was assessed by injecting a C7-C30 saturated alkane standard of 1000 µg/mL (Supelco). The data related to the assignment of the compounds are reported in Table 2S.

3. Statistical analysis

The quantity of the compounds was determined by the percentage of the total detected area (%TDA) in each chromatogram. The software Minitab 19 (v. 19.2) was applied to compare the classes and terpenoid compounds among clones, harvests and matrices. For the PCA, the data were expressed as the normalised chromatographic area (area of the volatile compound by the area of internal standard) using normalised data, log transformation and Pareto scaling, in addition to other multivariate analyses, applying MetaboAnalyst 5.0 software.

RESULTS

1. Volatile composition

Free volatile compounds were evaluated using HS-SPME/GC-MS for clones 809 and 76 cv. The compounds were divided into classes. Tables 1 and 2 show the percentage of %TDA of the following categories established according to chemical function, biosynthesis and yeast metabolism (Schwab *et al*., 2008; Ugliano and Henschke, 2009; Ilc *et al*., 2016): benzenoids, carbonyls, higher alcohols, esters, volatile fatty acids, volatile phenols, volatile sulphur, ether and terpenoids. For the purposes of providing a detailed evaluation, the terpenoid compounds are reported individually.

The berries of the two clones showed a higher quantity of carbonyl compounds (from 63.9 % to 79.5 % of TDA), followed by the higher alcohols. In 2017, the quantity of carbonyl was statistically different (one-way analysis of variance [ANOVA], $p < 0.05$, and Tukey test) between the clones; meanwhile, the higher alcohol quantities were equal. In contrast, the opposite was true in 2018 (Table 1). In Table 1 it can be seen that higher alcohol levels are greater in musts (from 26.2 % to 30.9 % of TDA), and that in both harvests, the amount was not statistically different ($p > 0.05$) between clones.

In Table 2, the higher quantity for the base and sparkling wines are esters (from 55.2 % to 71.1 % and from 57.4 % to 59.8 % respectively), followed by higher alcohols and volatile fatty acids. Regarding the base wines, the esters, higher alcohols, and fatty acids were statistically different ($p < 0.05$) between clones in 2017, whereas in 2018, the higher alcohols class was statistically different. For clone 76 in 2017, a low amount of esters (55.2 % of TDA) and high amount of volatile fatty acids (22.5 % of TDA) were found, followed by higher alcohols (11.7 % of TDA). In the case of sparkling wine, the main class of volatile compounds (esters) was not statistically different ($p > 0.05$) between clones.

2. Terpenoid compounds

The number and volume of terpenoid compounds biosynthesised in the two consecutive summer harvests of 2017 and 2018 are shown in Tables 1 and 2. Thirty-three terpenoids were identified in clone 809 in all matrices: 31 monoterpenoids, one sesquiterpenoid and one

TABLE 1. Volatile composition of grape berries and musts separated into classes and detailed for terpenoid compounds for two Chardonnay clones in vintages 2017 and 2018 in southeast Brazil (Caldas/MG).

		Grape berries				Musts			
		2017		2018		2017		2018	
Classes	VOCs	CL809	CL76	CL809	CL76	CL809	CL76	CL809	CL76
Benzenoids		1.51±0.06	2.22±0.18	4.15±0.49	7.27±0.39	0.75±0.01	0.47±0.05	0.59±0.03	2.32±0.33
Carbonyls		68.11±1.62	79.46±0.67	63.91±5.05	66.73±0.57	4.99±1.02	5.75±0.99	4.82±0.75	5.90±0.65
Esters		1.38±0.13	0.97±0.02	2.03±0.13	3.26±0.10	14.68±0.38	11.76±3.02	17.40±0.86	14.11±0.79
Higher Alcohols		11.38±0.59	12.41±0.76	15.09±1.19	21.39±0.47	26.20±0.34	26.91±4.16	30.89±1.67	30.63±5.34
Volatile Fatty Acids		2.74±0.25	3.52±0.21	nd	nd	2.46±0.28	1.67±0.32	3.50±0.14	5.51±1.74
Volatile Phenols		0.02±0.01	0.31±0.06	nd	nd	0.12±0.02	0.12±0.01	0.16±0.02	0.59±0.28
Volatile Sulphur		0.05±0.01	nd	nd	nd	0.09±0.02	0.15±0.04	0.02±0.00	nd
Others		0.02±0.0.01	0.02±0.01	nd	nd	nd	nd	nd	nd
Total Terpenoids		13.86±2.16	0.66±0.25	14.43±5.93	0.61±0.04	14.30±0.30	0.68±0.19	16.76±0.78	1.61±0.41
Monoterpenoid	ß-Thujene	nd	0.08±0.02	0.02±0.00	nd	nd	nd	nd	nd
Monoterpenoid	Limonene	0.10±0.03	0.51±0.0.23	0.23±0.08	nd	1.37±0.07	nd	1.17±0.12	0.08±0.01
Monoterpenoid	Eucalyptol	0.02±0.01	0.03±0.0.02	nd	nd	nd	0.18±0.05	nd	nd
Monoterpenoid	Cymene	nd	0.05±0.02	nd	nd	nd	nd	0.13±0.03	nd
Monoterpenoid	Tetrahydrolinalool	nd	nd	nd	0.07±0.0.03	nd	nd	nd	nd
Monoterpenoid	Linalool	10.33±1.87	nd	5.89±2.81	0.23±0.01	3.21±0.46	nd	4.29±0.37	0.19±0.07
Monoterpenoid	Menthol	nd	nd	0.05±0.01	0.07±0.00	nd	0.05±0.03	nd	nd
Monoterpenoid	Dihydromyrcenol	nd	nd	nd	nd	0.06±0.01	0.05±0.02	0.07±0.03	0.12±0.03
Monoterpenoid	ß-Myrcene	0.09±0.02	nd	0.66±0.20	nd	3.99±0.13	nd	2.73±0.09	0.20±0.03
Monoterpenoid	*trans*-ß-Ocimene	0.07±0.02	nd	nd	nd	1.25±0.06	nd	0.84±0.05	nd
Monoterpenoid	*cis*-ß-Ocimene	nd	nd	nd	nd	1.92±0.06	nd	1.02±0.02	nd
Monoterpenoid	(E,Z)-alloocimene	nd	nd	nd	nd	0.14±0.03	nd	0.16±0.09	nd
Monoterpenoid	ß-Phellandrene	nd	nd	0.07±0.04	nd	nd	nd	nd	nd
Monoterpenoid	α-Terpineol	0.70±0.15	nd	1.36±0.84	nd	0.33±0.02	nd	0.70±0.09	nd
Monoterpenoid	Citral	0.07±0.02	nd	0.21±0.07	nd	nd	nd	nd	nd
Monoterpenoid	Citronellol	0.52±0.06	nd	0.62±0.11	nd	nd	nd	0.83±0.17	nd
Monoterpenoid	Nerol	0.60±0.08	nd	1.35±0.40	nd	nd	nd	0.19±0.01	nd
Monoterpenoid	Nerol oxide	nd	nd	nd	nd	0.78±0.03	nd	1.39±0.07	nd
Monoterpenoid	Geraniol	1.20±0.21	nd	2.36±0.73	nd	nd	nd	0.43±0.10	nd
Monoterpenoid	*trans*-Linalool oxide (furanoid)	nd	nd	0.91±0.51	nd	nd	nd	0.71±0.02	nd
Monoterpenoid	Geranic acid	0.16±0.06	nd	0.44±0.25	nd	0.11±0.03	nd	0.20±0.05	nd
Monoterpenoid	*trans*-Geranic acid methyl ester	nd	nd	nd	nd	0.03±0.01	nd	0.12±0.02	nd
Monoterpenoid	*cis*-Linalool oxide	nd	nd	nd	nd	0.61±0.06	nd	nd	nd
Monoterpenoid	Hotrienol	nd	nd	nd	nd	nd	nd	0.33±0.01	nd
Monoterpenoid	Geranylacetone	nd	nd	0.16±0.06	nd	0.24±0.04	nd	0.92±0.17	nd
Sesquiterpenoid	Farnesene	nd	nd	0.10±0.01	0.07±0.03	nd	nd	nd	nd
C13-Norisoprenoid	ß-damascenone	nd	nd	nd	0.17±0.04	0.25±0.01	0.41±0.13	0.54±0.06	1.01±0.29

*VOCs: volatile organic compounds; CL: Clone; Nd: not detected.

TABLE 2. Volatile composition of base and sparkling wines separated into classes and detailed for terpenoid compounds for two Chardonnay clones in vintages 2017 and 2018 in southeast Brazil (Caldas/MG).

		Base wines				Sparkling wines			
		2017		2018		2017		2018	
Classes	VOCs	CL809	CL76	CL809	CL76	CL809	CL76	CL809	CL76
Benzenoids		0.05±0.01	0.07±0.01	0.17±0.01	0.10±0.03	0.15±0.01	0.18±0.01	0.63±0.03	0.22±0.01
Carbonyls		0.13±0.03	0.06±0.01	0.06±0.00	0.05±0.01	0.24±0.01	0.44±0.05	0.43±0.01	0.38±0.02
Esters		71.14±1.29	55.23±4.48	70.22±0.69	68.79±1.16	59.82±3.05	59.13±2.66	59.06±0.88	57.44±1.15
Higher Alcohols		8.34±0.23	11.73±0.11	5.88±0.22	7.11±0.28	7.93±0.24	10.08±0.71	8.46±0.37	10.12±0.20
Volatile Fatty Acids		8.35±0.54	22.52±1.29	9.10±0.67	9.36±1.43	8.71±0.36	7.64±0.87	9.92±0.14	11.70±0.0.31
Volatile Phenols		nd	0.19±0.08	0.10±0.02	0.15±0.20	0.17±0.05	0.07±0.09	0.01±0.00	0.03±0.00
Volatile Sulphur		nd	0.04±0.01	0.02±0.01	0.02±0.00	0.01±0.00	0.01±0.0.01	0.03±0.01	0.07±0.01
Ether		0.04±0.01	0.02±0.00	0.01±0.00	0.01±0.00	0.02±0.00	0.01±0.00	0.02±0.01	0.01±0.00
Total Terpenoids		1.43±0.09	0.43±0.05	1.01±0.04	0.20±0.01	0.77±0.06	0.12±0.04	1.19±0.09	0.07±0.02
Monoterpenoid	Limonene	0.02±0.00	nd	0.02±0.00	nd	0.02±0.01	nd	nd	nd
Monoterpenoid	Linalool	0.59±0.01	0.03±0.00	0.37±0.00	0.02±0.00	0.12±0.01	nd	0.30±0.02	0.01±0.00
Monoterpenoid	Menthol	nd	nd	nd	nd	nd	nd	nd	nd
Monoterpenoid	α-Terpineol	0.06±0.01	nd	0.04±0.01	0.01±0.00	0.22±0.01	0.02±0.02	0.31±0.02	0.02±0.01
Monoterpenoid	Citronellol	0.15±0.02	0.10±0.02	0.13±0.01	0.04±0.00	0.03±0.00	nd	0.05±0.01	nd
Monoterpenoid	ß-Myrcene	0.06±0.01	nd	0.02±0.00	nd	0.01±0.00	nd	0.03±0.00	nd
Monoterpenoid	Nerol	nd	nd	0.02±0.00	nd	nd	nd	nd	nd
Monoterpenoid	Nerol oxide	0.07±0.00	nd	0.07±0.00	nd	0.11±0.01	nd	0.15±0.02	nd
Monoterpenoid	Hotrienol	0.03±0.01	nd	0.04±0.00	nd	0.04±0.01	nd	0.06±0.01	nd
Monoterpenoid	Geranic acid	0.01±0.00	nd	nd	nd	0.01±0.01	nd	nd	nd
Monoterpenoid	Linaloyl oxide	nd	nd	nd	nd	0.03±0.01	nd	0.03±0.00	nd
Monoterpenoid	(Z)-Dehydroxylinalool oxide	nd	nd	nd	nd	0.03±0.01	nd	0.06±0.02	nd
Monoterpenoid	*trans*-Linalool oxide (furanoid)	nd	nd	nd	nd	0.05±0.01	nd	0.10±0.04	nd
Monoterpenoid	L-Rose oxide	nd	nd	nd	nd	nd	nd	0.01±0.01	nd
Monoterpenoid	Isoterpinolene	nd	nd	0.01±0.01	nd	nd	nd	nd	nd
Monoterpenoid	Citronellyl acetate	nd	nd	0.07±0.01	0.02±0.01	nd	nd	nd	nd
Monoterpenoid	Geranyl acetate	nd	nd	0.02±0.01	0.01±0.00	nd	nd	nd	nd
Monoterpenoid	Geranylacetone	0.28±0.12	0.09±0.05	0.04±0.03	0.01±0.00	0.06±0.03	0.07±0.05	0.02±0.02	001±0.00
C13-Norisoprenoid	ß-damascenone	0.16±0.02	0.20±0.01	0.17±0.00	0.11±0.11	0.03±0.00	0.03±0.01	0.06±0.00	0.03±0.01

*VOCs: volatile organic compounds; CL: clone; Nd: not detected.

C13-norisoprenoid. Clone 809 showed double the number of monoterpenoids compared to clone 76.

In the berries, clone 809 showed 13.9 % and 14.4 % of terpenoid compounds in 2017 and 2018 respectively, while for clone 76, the TDA was 0.7 % and 0.6 % for each season (Table 1); this reflects the biosynthesis of a greater amount of monoterpenoids by clone 809 - mainly linalool, α-terpineol, citronellol, nerol and geraniol.

In post-*débourbage* musts (Table 1), the TDA of terpenes was 14.3 % and 16.8 % in 2017 and 2018 respectively for clone 809, and 0.7 % and 1.6 % for clone 76. The higher quantity of free terpenes in the must is not statistically different (one-way ANOVA, $p > 0.05$, and Tukey test) from the berries in either vintage. However, in the case of clone 809, a change in the profile of the compounds is observed for these matrices, with a higher percentahge of TDA of the following compounds for the 2017 and 2018 vintages

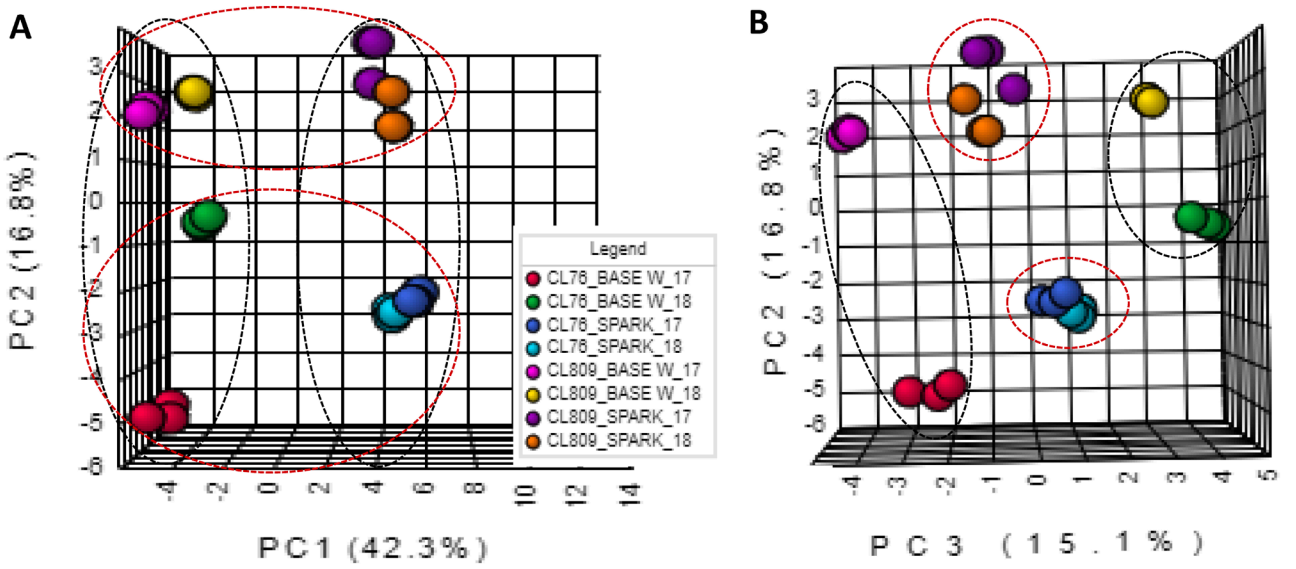

FIGURE 1. Principal component analysis (PCA) for volatile compounds identified in the base (BASE W) and sparkling wines (SPARK) of Chardonnay clones (CL) 76 and 809 in two consecutive vintages in southeast Brazil (Caldas/MG).

Figure 1A: PC1 vs PC2, clusters differentiating the process step (left: base wine and right: sparkling wine) and the clones (bottom: clone 76 and top: clone 809). Figure 1B: Four clusters in PC3 – left and right sides the base wines of 2017 and 2018, respectively, and in the middle top: the sparkling wines of clone 809, and bottom: sparkling wines of clone 76.

respectively: ß-myrcene (4.0 % and 2.7 %), linalool (3.2 % and 4.3 %), limonene (1.4 % and 1.2 %), trans-ß-ocimene (1.2 % and 0.8 %), cis-ß-ocimene (1.9 % and 1.0 %) and oxidative metabolites of linalool (0.61 % and 0.71 %) (Luan *et al*., 2006; Ilc *et al*., 2016).

Regarding the total terpenoids in the stabilised base wine (Table 2), clone 809 contained 1.4 % in 2017 and 1.0 % in 2018, whereas clone 76 contained 0.4 % and 0.2 %. The percentage of TDA of this class of compounds is statistically different between the two clones for both vintages ($p < 0.05$), reflecting the difference between an aromatic and non-aromatic clone. In the base wines of clone 809, the compounds in greater quantity in each vintage (2017 and 2018) are linalool (0.6 % and 0.4 %) and citronellol (0.1 % and 0.1 %). In vintage 2018, monoterpene esters (citronellyl acetate and geranyl acetate) were observed.

The sparkling wines of clone 809 contained 0.8 % and 1.2 % of terpenoid compounds in 2017 and 2018 respectively (Table 2); meanwhile, clone 76 contained 0.1 % and <0.1 % in 2017 and 2018 respectively. The compounds in the highest quantities were the alcohols of monoterpenes, α-terpineol (0.2 % in 2017 and 0.3 % in 2018) and linalool (0.1 % and 0.3 %).

3. Multivariate analysis

For the multivariate analysis, 84 and 90 compounds in the base wines and 89 and 93 volatiles in the sparkling wines were identified in 2017 and 2018 vintages respectively.

In total, 121 variables were noted and considered significant using Tukey´s test ($p < 0.05$). It was thus possible to carry out the PCA, in which the ratio of the areas of the free compounds (compound area/internal standard area) was determined for clones 76 and 809. The total variance of the data for the three principal components was 74.2 % (Figure 1A and 1B). PC1 differentiated the elaboration stage by 42.3 % (base vs sparkling wine), and PC2 discriminated the clones used to prepare the base and sparkling wines (clone 809 vs 76; PC2: 16.8 %). For the third component, there were four clusters; the base wines were differentiated by the vintage (2017 base wine vs 2018 base wine). However, the sparkling wines were clustered by their clones (Figure 1B). The most significant volatile compounds in the sparkling wines of clone 809 that differentiated them in both vintages were α-terpineol, linalool, ß-myrcene, hotrienol, nerol oxide and limonene.

DISCUSSION

This is the first study to be conducted on the characterisation of volatile composition of Chardonnay clone 809 compared to clone 76 in Serra da Mantiqueira, southeast Brazil (Caldas, MG), a region where Chardonnay clone 76 is generally used in commercial vineyards A mutation in the enzyme 1-deoxy-D-xylose-5-synthase catalyses the production of monoterpenoid compounds in the Chardonnay clone 809, which may impart a more floral and fruity character to the wine.

Differences in the volatile composition of the grapes were found between harvests; this can be explained by the variability of the climatic conditions, which depend on the season, temperature, rainfall, thermal amplitude and solar radiation, and which affect enzymatic activity, gene expression and primary and secondary metabolism (Drappier *et al*., 2017; Luo *et al*., 2019; van Leeuwen *et al*., 2020).

Regarding the volatile composition in the other steps, statistical differences were found between the main

compounds (esters, volatile fatty acids and higher alcohols) in the base wine between clone and season, which can be explained by the lack of esterification (volatile fatty acids with ethanol or higher alcohol with acetyl-CoA) in 2017 due to poor enzymatic activity, ethanol amount or ester hydrolysis due to temperature. However, the volume of esters in the sparkling wines for both clones was not found to be statistically different, demosntrating that an equilibrium was reached by the main volatile compound class in the final product.

The percentage of TDA of monoterpenoid compounds in clone 809 (aromatic clone) grapes is higher (Table 1), on the other hand the results for clone 76 corroborate data reported in the literature (Duchêne *et al*., 2009; Luo *et al*., 2019); this demonstrates that Chardonnay is a cultivar that contains a low quantity of these compounds, with most of its clones being considered non-aromatic. The scents produced by the main monoterpenoid compounds found in the berries in both seasons (e.g., linalool, α-terpineol, citronellol, nerol and geraniol) are floral, sweet and citric. An evaluation could be carried out on the viticultural practices that favour the synthesis of terpenoid compounds, such as exposing bunches to light (Friedel *et al*., 2016; van Leeuwen *et al*., 2020), and which consequently enhance the wine *bouquet*.

The difference in free monoterpenoid compounds in the musts comparing the harvests for clone 809 may be due to the influence of environmental conditions (light and temperature) on enzymatic activity and gene expression, as previously described regarding the volatile composition of berries.

The reduction in terpenoid content from the must to base wine (13.3 % TDA decrease) in both seasons can be explained by the base wine's tartaric and protein stabilisation stages. Using bentonite for protein clarification can reduce aromatic compounds in sparkling wine production (Ubeda *et al*., 2021). In addition to eliminating proteins, bentonite removes aromatic compounds; it is not selective when used as a clarifier to eliminate proteins or as an adjuvant in the remuage process. Additionally, Slaghenaufi *et al*. (2020) found that these compounds can be adsorbed by yeast cells due to their polarity and thus be lost. Further studies should be conducted on how to avoid the loss of terpenoid compounds during sparkling wine production (fining, adjuvant and adsorption in dead yeast cell walls).

In 2018, monoterpene esters were evaluated in the base wine of both clones. Although yeasts from the *Saccharomyces* genus are unable to biosynthesise terpene compounds due to the lack of terpene synthase enzymes (González-Barreiro *et al*., 2015), monoterpenol compounds can undergo biotransformation catalysis through acetylation, forming terpinyl acetates (citronellyl acetate from β-citronellol, and geranyl acetate from geraniol), as demonstrated by Slaghenaufi *et al*. (2020) using the *Saccharomyces cerevisiae* strain.

Monoterpene alcohols can be converted by reduction, oxidation, hydroxylation, isomerisation, translocation and cyclicisation (King and Dickinson, 2000; Slaghenaufi *et al*., 2020) into other terpenoid compounds; for example, geraniol can be converted into citronellol or linalool. In this study, the lack of geraniol in the base and sparkling wines may be one explanation for the conversion to other monoterpenoids, but further studies are necessary to confirm this. Although this conversion can influence the organoleptic properties and the intensity of wines (King and Dickinson, 2000), geraniol (antibacterial and cytotoxic) conversion is a way the yeast enzymes detoxify the media (Slaghenaufi *et al*., 2020).

Regarding the multivariate analysis, Figure 1B suggests that, regardless of the vintage, the sparkling wines in the third component had an influence of the clones. Thus, even after two fermentations, the rose and sweet character of the sparkling wine produced with clone 809 may be due to the following monoterpenoid compounds: α-terpineol, linalool, ß-myrcene, hotrienol, nerol oxide and limonene. The alcohols of monoterpenes are potent odorants (González-Barreiro *et al*., 2015) which may confer floral, fruity, sweet and pungent aromas (Burdock, 2010) to the wine.

CONCLUSION

The data suggest that clone 809 expresses a varietal characteristic due to the monoterpenoid compounds in the berries and sparkling wines, differing from clone 76 in terms of quantities of terpenoid compounds. Some of these compounds discriminated the sparkling wines made with Chardonnay clone 809 in the multivariate analysis, primarily monoterpene alcohols, which are considered fairly odoriferous. Among the monoterpene alcohols, compounds such as linalool, α-terpineol and hotrienol are present, which have flowery, fruity and sweet aromas.

This preliminary study in the Serra da Mantiqueira region could be a driver for further experiments on improving the aromatic potential of clone 809 in the vineyard and during vinification, applying, for example, agricultural techniques (e.g., training systems and exposure of bunches and leaves to solar radiation) and different vinification processes (e.g., use of other yeasts, develop the process in such way that the loss of terpenoid compounds is reduced). Additionally, extraction methods could be applied to evaluate the free and bound forms of the volatile compounds and to quantify them in order to determine the precise potential of the use of this clone for sparkling wines.

ACKNOWLEDGEMENTS

Laboratory infrastructure: the University of São Paulo and FAPESP (Grant number: 2013/07914-8); Vineyard implementation: FAPEMIG (Grant number: CAG-PPM-00301-16) and Vitacea Bra.

This investigation was supported by laboratory practices (infrastructure, equipment and consumables) and the vineyard implementation in the agronomical centre. Thanks to all EPAMIG staff who helped in the vineyards and winery.

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