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Protection against oxygen in the vinification of the red grape Nerello Mascalese affects volatile organic compounds profile

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ABSTRACT

We hypothesised that the protection against oxygen (PAO) of must and wine during vinification by avoiding contact with air can preserve the primary volatile organic compounds (VOCs) of Nerello Mascalese. PAO was performed in two seasons (2020 and 2021) using carbon dioxide pellets ($CO_{2,s}$) and gas ($CO_{2,g}$), inactivated yeasts and ascorbic acid during fermentation; the control wine was made via traditional vinification without adding the aforementioned components. During fermentation, the two winemaking processes mainly differed in terms of the application of aeration during maceration/fermentation in the pump-over and délestage activities, and the care taken to avoid contact with oxygen during racking. In both years, higher concentrations of polyphenols and total anthocyanins were found in the PAO wine (about 16 % and 20 % respectively). The concentrations of nerol, citronellol and geraniol were significantly higher in the PAO wine in both seasons, albeit with small differences which affected the sensory evaluation. The free/bound terpenes ratio was 0.35 (PAO) and 0.55 (Control). Bound C_{13}-norisoprenoids contents were higher than the free ones; in the PAO wine, free 3-oxo-α-ionol and bound vomifoliol concentrations were slightly higher than in the control wine. Thiols were measured in Nerello for the first time. In 2020 in particular, sulfanylhexan-1-ol was present in larger amounts in the PAO wine. Applying PAO in the production of Nerello Mascalese modified certain VOCs, as well as the overall free/bound ratio due to the increase in bound VOCs, thus influencing the aroma of the wine.

KEYWORDS: Winemaking, Nerello Mascalese, VOCs, thiols, terpenes, C13-norisoprenoids

INTRODUCTION

Italy is a country where mountains and hills represent 65 % of the territory, with hills representing over half this figure. It is on the fault line between the African and European tectonic plates with several inactive and active volcanoes. Viticulture is spread almost all over Italy, but under highly differing climatic conditions. This extreme diversity, which is also emphasised by the multiplicity of the traditional cultivated germplasm (D'Onofrio *et al*., 2021), forms the basis of "varietal oenology". The objective of "varietal oenology" is to produce diverse and quality wine via the optimisation and expression of grape variety attributes, which are, in turn, an expression of the territory (the environment, vine training system, and vineyard management). Reductive winemaking has always been associated with white wine production (Baiano *et al*., 2016), being particularly suitable for white grape varieties that are rich in varietal aromas and sensitive to oxidation (Ardilouze, 2006), such as Sauvignon blanc, Colombard, Petit Manseng, Chenin blanc and Gewürztraminer; however, it is also applied on red wines, such as Grenache, Cabernet franc and Merlot, which are used for producing rosé wines. Reductive winemaking is mainly carried out by limiting the contact of must with oxygen and by adding SO_2 coupled with ascorbic acid. Further developments include the hyper-reductive winemaking technique, involving the use of gases like CO_2 and N_2, and prefermentative cryomaceration, which aims to protect grapes against oxidation during the crushing phase, and thus to preserve the primary VOCs of grapes (Baiano *et al*., 2016). Notwithstanding the objective to preserve the primary aromatic compounds, the observation of the effect of reductive winemaking on red grape was aimed also at protecting the polyphenols (Antonelli *et al*., 2010; Baiano *et al*., 2012). The "reductive" character of wine is of great concern in reductive winemaking, which is mainly related to the development of fermentation-derived low molecular volatile sulfur compounds (LMVSCs), such as hydrogen sulfide, methanethiol and dimethyl sulfide (Bekker *et al*., 2016; Kreitman *et al*., 2017).

Nerello Mascalese is a red grape variety from southern Italy, with optimal growing conditions in the Etna Volcano area. Genetic studies have proved that Nerello originates from Sangiovese and Mantonico bianco varieties (D'Onofrio *et al*., 2021). The wines are characterised by low polyphenol content (about 2000 mg/L), as well as low anthocyanin content (around 125 mg/L; Giacosa *et al*., 2021). In term of VOCs in wine, Ansaldi *et al*. (2014) found the highest levels of terpenes to be trans-8-hydroxylinalool and geraniol, and the highest levels of C_{13}-norisoprenoids to be 3-oxo-α-ionol and vomifoliol. The same authors concluded that the low content of grape aroma compounds was not favourable for the production of wine based on such grape characteristics. To confirm this assumption, Sparacio *et al*. (2009) carried out cryomaceration on Nerello Mascalese grape to produce wine and did not find any significant differences in terms of VOCs between the control and the cryomacerated sample.

Taking into account these observations, our hypothesis in this research work was that protection of must against oxygen (PAO) throughout the vinification process results in the expression of grape VOCs. For this reason, the vinification process of the test wine (PAO) was different from that of the control wine, but the yeasts and fermentation activators were the same.

MATERIALS AND METHODS

1. Grapes and vinification

The experimental vineyard was located in the Cottanera Winery (Castiglione di Sicilia, CT), in the district of Randazzo, in the northern area of Etna. Grape clusters of Nerello Mascalese (*Vitis vinifera* L.) were harvested from the same vineyard in the seasons of 2020 and 2021. The vineyard had a north/south exposure and an elevation of 700 m. The vines were 15 years old, 2.2 m x 0.9 m distance between rows, trained to a "cordon spur", and grafted onto rootstock R108 in the 2020 and 2021 seasons. The characteristics of the grape at harvest are given in Table 1

Sound and uniformly coloured bunches were selected and manually harvested and placed in perforated crates (15 ± 1 kg) at the end of October in both study years, when temperature was around 20 ± 2 °C during the day and 10 ± 1 °C at night. No additives were added to the grape clusters. The grapes were taken to the cellar (20 min drive away). The grapes, which had a temperature of 21 ± 1 °C, were tossed in the receiving hopper (volume of about 20 m^3) that evenly fed the grape clusters into the destemmer-crusher. The distance between the rollers of the crusher was regulated according to berry diameter in order to crush the berries but leave the skin intact. The grapes then fell into the crusher, which crushed the berries without crushing the green parts (leaves and stems), skins and seeds. The stainless-steel crusher was a horizontal closed cylinder, with dull propeller-like blades for separating the stems from the berries as they

TABLE 1. Results of the main analyses of the grape juice at the time of harvest. Data are the mean (± SD) of five juice analyses of berries from different bunches. No statistical differences were found between the two years in terms of the values of each parameter.

Season	Berry weight (g)	pH	°Babo	Titratable acidity (g/L expressed as tartaric acid)	Malic acid (g/L)
2020	2.1 ± 0.1	3.32 ± 0.02	21.95 ± 0.97	6.7 ± 0.2	2.1 ± 0.2
2021	1.9 ± 0.3	3.26 ± 0.04	21.05 ± 0.85	6.2 ± 0.4	2.4 ± 0.2

passed through, without breaking the stems. Before receiving the clusters, the receiving hopper and the destemmer-crusher had been cooled using enough dry ice to create a deep fog in the hopper. When all the dry ice had sublimated and the CO_{2gas} fog had filled the hopper, the grape clusters were unloaded into the hopper, where they immediately cooled (to approx. 8 °C) without coming into contact with the dry ice. The concentration of oxygen and carbon dioxide gas in the empty hopper (measured with an oxygen/carbon dioxide sensor (Helpy, Marvil srl, Bozen, Italy)), was not homogenous: while no oxygen was recorded at the bottom of the tank, it gradually increased towards the top to 8 ± 2 %. For this reason, to avoid too much contact with oxygen the hopper was not loaded right up to the top. In the crusher, the oxygen concentration was higher due to the rotation of the blade (11 ± 2 %). The crushed clusters then passed through a tube-in-tube heat exchanger to guarantee cooling at 8 – 10 °C, the temperature required for cold maceration (for 24 h). At this point, two parallel procedures were carried out: protection against oxygen (PAO) and the preparation of the control (following the winery protocol).

PAO: All the pumps, the heat exchanger and pipes used in must handling were filled with $CO_{2,g}$ to avoid contact with oxygen. Particular attention was paid to preventing oxygen from entering the pipes when one batch of grapes was unloaded and before receiving the new batch, by filling them with $CO_{2,gas}$. The maceration/fermentation vessel (100 hL) was filled with $CO_{2,gas}$ via the bottom valve, ensuring the top valve of the vessel was open (1 kg of $CO_{2,g}$ to fill approx. 0.5 m^3); the latter valve was closed when the saturation point was reached (measured using a gas analyser). The vessel was then filled with must (both solids and liquid) via the bottom using a piston pump (Polsinelli Enologia srl, Isola del Liri, Italy). After vessel filling, 0.2 g/kg inactivated yeast Fresh Aroma (Laffort, Bordeaux, France), 0.04 g/kg of ascorbic acid, 0.08 g/kg of potassium metabisulfite (MBK), and 0.15 g/kg of BO213 yeasts (Laffort, Bordeaux, France) were added to the must. The following pump-overs were performed, based on the percentage of solids (from the grape):

Day 1: 3 pump-over of 40 ± 5 % of grape (closed system without any contact with air)

Day 2: 2 pump-over of 40 ± 5 % of grape (closed system, without any contact with air)

Day 3: 2 pump-over of 40 ± 5 % of grape (closed system, without any contact with air)

Day 4: délestage (1/3 of the mass in air, by using the Venturi tube to inject air to 1/3 of the mass during the refilling of the fermentation vessel)

Day 5: 1 pump-over of 40 ± 5 % of grape (closed system, without any contact with air)

From the 6th day: 1 pump-over of 7.5 % of grape (closed system, without any contact with air).

The control: vessel was filled with must (both solids and liquid) without taking measures to prevent contact with oxygen in the pipes, pumps and vessel; thus, the vessel contained air before it was filled with the must. To the must 0.15 g/kg of BO213 yeasts (Laffort, Bordeaux, France) and 0.07 g/kg of MBK (a lower concentration was used than in the PAO due to less oxygen protection being required) was added. The must was then transferred to maceration/fermentation tank (100 hL). Neither inactivated yeasts nor ascorbic acid were added, as the winery's normal protocol was being followed. The following pump-overs were performed based on the percentage of solids (from the grape):

Day 1: 3 pump-over of 40 ± 5 % of grape (closed system)

Day 2: 2 pump-over of 40 % of grape (closed system)

Day 3: 2 pump-over of 40 % of grape (closed system)

Day 4: délestage (100 % of mass in air)

Day 5: 1 pump-over of 40 % of grape (air)

From the 6th day: 1 pump-over of 7.5 % of grape (in air using the Venturi tube as described above).

In both the PAO and control vessels, the must temperature before fermentation was kept at 10 ± 1 °C for the first 4 days and then every day it was raised, starting from 16 °C and finishing at 28 ± 1 °C after 8 days (in 2020) and 9 days (in 2021) when both wines were completely dry (i.e., no residual sugars were measured). After the fermentation process, the racking of the PAO wine was carried out without any contact with oxygen by saturating the pump and the pipes with $CO_{2,g}$, while the control wine racking process was carried out without dry ice.

Malolactic fermentation (MLF) was started inside the stainless-steel vessels. For the PAO sample, the same procedure used to avoid contact with oxygen when filling the vessel for maceration/fermentation was adopted. Once the vessel was filled with wine, the cask fennels were placed on the top openings of both vessels (PAO and control), and after the addition of 2 % of lees from other vessels, MLF was triggered. MLF ended in both wines after 2 months at room temperature (17 – 20 °C) in the cellar, when potassium metabisulfite (MBK) was added to obtain 25 mg/L of final free SO_2. The wines were kept in stainless steel vessels which had been completely filled.

2. Chemical analyses

The average was determined from 80 clusters of berries and berry sampling was carried out by picking berries from the top, middle and bottom of each cluster. The berries were crushed in the winery and homogenised with a commercial homogeniser, and the main analyses at harvest (Table 1) were carried out using OIV methods (2009). Wine after MLF was shipped to Pisa and analysed in the DAFE laboratory of the University of Pisa by a calibrated Fourier transform infrared WineScan™ FT 120 (Foss Analytics, Hillerod, Denmark) for the following oenological parameters in triplicate: sugars (g/L), pH, titratable acidity (tartaric acid g/L), volatile acidity (g/L acetic acid), malic acid (g/L),

tartaric acid (g/L), citric acid (g/L), total extract (g/L), ashes (g/L), glycerol (g/L), gluconic acid (g/L) total anthocyanins (mg/L malvidin), and total polyphenols (mg/L gallic acid). Each sample was analysed in triplicate; five 375 mL bottles were subjected to three WineScan™ analyses each season. The accuracy of the WineScan™ analyses was validated by performing destructive analyses using traditional methods during calibration before starting the readings, followed by OIV methods (2009).

The analyses of the wine for VOCs (three 375 mL bottles) was performed by using the gas chromatographic method as described by Bellincontro *et al*. (2016). Aroma compounds were extracted from the wines by solid phase extraction (SPE); gas chromatographic analysis was carried out using an Agilent 7890A gas chromatograph (Agilent Technology, Santa Clara, Ca, USA) and an Agilent 5975C quadrupole Mass Spectrometer (MS). The carrier gas was helium at a constant flow rate of 1 mL/min. The capillary column was an HP-Innowax (30 m length, 0.25 mm i.d., 0.25 mm film thickness) from Agilent. The column oven was programmed as follows: starting temperature of 30 °C, then an increaseof 30 °C/min to 60 °C, 2 °C/min to 190 °C and finally 5 °C/min to 230 °C. The MS detector scanned within a mass range of m/z 30–450. Volatile compounds were tentatively identified by comparing the mass spectra with those available in the data system library (NIST 08, National Institute of Standards and Technology, Gaithersburg, MD, 2008) and using published retention indices. The characterisation was considered positive when a volatile compound with a probability of > 70 % was identified in at least three independent samples. When possible, the identity of the compounds was further confirmed by comparing the retention times with authentic standards reported here: isoamyl alcohol, (E)-furan linalool oxide, (Z)-furan linalool oxide, 1-Hexanol, 1-Pentanol, *trans*-2-Hexen-1-ol, 2-Hexenal, 2-Phenylethanol, acetophenone, α-citral, ß-citral, *trans*-3-Hexen-1-ol, *cis*-3-Hexen-1-ol, α-terpineol, ß-citronellol, benzaldehyde, benzyl alcohol, α-ionol, 1-octanol, guaiacol, vanillin, eugenol, geranic acid, geraniol, acetovanillone, homovanillic alcohol, linalool, methyl salicylate, myrtenol, nerol, farnesol, *trans*-cinnamic acid. All reagents and standards were purchased from Sigma-Aldrich (St Louis, MO). Quantification was carried out by comparing the peak area of each compound to that of the internal standard (1-heptanol). Volatile thiols (in detail, 3-sulfanylhexanol, 4-methyl-4-sulfanyl pentan-2-one, 3-sulfanylhexyl acetate, ethyl-2-sulfanyl acetate, 2-furanmethanethiol and benzenemethanethiol) were quantified in wines by gas chromatography–tandem mass spectrometry (GC-MS/MS) following an analytical procedure adapted from Thibon *et al*. (2015). 50 µL of internal standard mix containing 6-sulfanylhexanol (6SH, 500 µg/L, EtOH), 4-methoxy-2-methyl-2-sulfanylbutan (MMSB, 500 µg/L), ethyl maltol (EM, 1 mg/L, EtOH) and 3-octanol (1 mg/L, EtOH) was added to a sample of 20 mL of wine. The samples were percolated through a conditioned SPE column (HR-X, 6 mL, 500 mg, Macherey Nagel, France). Then, the SPE columns were rinsed twice with 2 mL of hydro-alcoholic solution (10 %) and the aromatic compounds were eluted with 3 mL of pentane/dichloromethane (50/50; v/v), followed by 3 mL of dichloromethane/methanol (95/5; v/v). The obtained organic phases were blended, dried over anhydrous sodium sulphate and then concentrated to 150 µL under a nitrogen stream. Here, only data from the 2020 season is reported, because, unfortunately, in 2021, the samples were misplaced during shipping to Bordeaux University and it was not possible to obtain replacement samples due to the wine having been blended in the winery.

As already mentioned, all the chemical analyses were performed on samples of wine from three 375 mL dark glass bottles after homogenising the wine mass inside the vessel before taking the sample to ensure the uniformity of the wine sample. The bottles were filled with dry ice and, after sublimation, with wine; they were then capped with a natural cork. All the analyses were performed within two months of bottling. The bottles were kept at room temperature (20 ± 2 °C).

3. Sensory analysis

The descriptive sensory analysis of the wine was carried out in the tasting room of the winery by a trained panel of 10 experts comprising winemakers, wine journalists, researchers and winery owners; all of them had expertise in tasting Nerello Mascalese. In a single session, they smelt and tasted PAO and control wines each from three unlabelled dark glass bottles (750 mL each and which had been collected from the tank as described in the Materials and Methods Section). The wines were presented to the assessors in wine-tasting glasses (ISO 3591-1997) at room temperature (22 ± 1 °C). After sniffing and tasting, the assessors used a 10 cm unstructured scale by Cejudo-Bastante *et al*. (2011) (modified) to evaluate the wine. The reported attributes had been previously identified and selected by the panelists drawing on their own experience of Nerello Mascalese wine tasting.

4. Statistics

ANOVA was applied to the date and significance was evaluated by comparing mean values with Tukey's test ($p < 0.05$) using GRAPHPAD PRISM 3.05 (GraphPad Software, La Jolla, CA, USA).

RESULTS AND DISCUSSION

The procedure carried out in the winery for preventing the oxygen from coming into contact with the grape, must and wine did not modify the fermentation process, which lasted 8 days (2020) and 9 days (2021) for both wines. The values for theoenochemical parameters at the end of malolactic fermentation of the wine are given in Table 2.

Alcohol content was similar in the two samples in both seasons. No significant differences were found in terms of pH; titratable acidity was higher in the 2020 season, but no differences between the two samples (PAO and Control) were observed. In contrast, volatile acidity was significantly higher in the 2021 season, but no differences were found between the two samples. In both seasons, the PAO wines had significantly higher concentrations of total polyphenols

TABLE 2. Results of main analyses of the two wine samples (protection against oxygen (PAO) and traditional vinification (Control) at the end of MLF (2020 and 2021 seasons). Only the values followed by different letters (each parameter) are statistically significantly different ($P < 0.05$).

Season	Alcohol (v/v)		pH		Titratable acidity (g/L expressed as tartaric acid)		Volatile acidity (g/L)		Free SO_2 (mg/L)		Total polyphenols (mg/L)		Total anthocyanins (mg/L)	
	PAO	Control	PAO	Control	PAO	Control	PAO	Control	PAO	Control	PAO	Control	PAO	Control
2020	14.2 ± 0.2	14.3 ± 0.1	3.60 ± 0.05	3.63 ± 0.05	6.0 ± 0.2^{a}	6.0 ± 0.1^{a}	0.36 ± 0.05^{b}	0.30 ± 0.03^{b}	24 ± 1	26 ± 1	2525 ± 76^{a}	2105 ± 78^{b}	350 ± 12^{a}	282 ± 22^{b}
2021	14.0 ± 0.3	14.3 ± 0.1	3.70 ± 0.06	3.67 ± 0.04	5.0 ± 0.3^{b}	4.9 ± 0.3^{b}	0.58 ± 0.04^{a}	0.55 ± 0.05^{a}	26 ± 1	26 ± 1	2476 ± 110^{a}	2089 ± 80^{b}	335 ± 10^{a}	270 ± 10^{b}

and total anthocyanins, which is likely due to less oxidation occurring (Antonelli *et al*., 2010) as a result of the PAO process from grape to wine in the winery.

In this section, the VOCs related to primary aroma (grape) have been separated from the VOCs (in particular esters and alcohols) mainly related to the fermentation process, as well as those related to sulfur compounds.

Table 3 shows the VOCs that we assumed to be mainly derived from grapes.

The total amount of free terpenes was higher in the control wines than in the PAO wines in both seasons due to a significantly higher content of exo-2-hydroxy-1,8-cineole. This compound is an alcohol present in several types of herb either as it is or as acetate, and in grape it is classified as belonging to the α-terpineol class (D'Onofrio *et al*., 2016). Exo-2-hydroxy-1,8-cineole is an oxygenated form of 1,8-cineole (Stok *et al*., 2016), or of α-terpineol as suggested by Bitteur *et al*. (1990), who discovered it for the first time in Sauvignon grape; meanwhile, Lamorte *et al*. (2008) detected a high concentration of the same VOC (about 30 µg/L) in Falanghina grape. This molecule can contribute to wine aroma with notes of blackcurrant. Being an oxygenated form, the higher content of exo-2-hydroxy-1,8-cineole in the control wine may be due to an oxidative process occurring during the vinification process. The PAO wines showed slightly higher contents of free nerol, citronellol and geraniol; these results were significant. Nerol and geraniol are very sensitive to oxidation upon which they transform into α-terpineol (Baron *et al*., 2017), whose concentrations were similar in the two samples, indicating that less oxidation had occurred in the PAO sample. Low levels of oxide C and D (epoxylinalool) were measured without any significant differences. Regarding the concentrations of free nor-isoprenoids in both samples (Table 3), the highest values were obtained for 3-oxo-α-ionol, followed by vomifoliol, with a significant difference between the two years, but not between the samples from the same year. Regarding the free C6 compounds), total content was found to be slightly higher in the PAO wines in both seasons, with concentrations of 1-hexanol 100-fold that of the other C6 compounds (Table 3), especially in the 2020 season. The Green Leaf Volatiles (GLVs) - thus named because they give wine a green grass aroma - are short-chain (C_6/C_9) acyclic aldehydes, alcohols and esters that form as a result of catalysis by LOX, hydroperoxide lyases (HPLs) and ADH, and they are the main source of green aroma in grapes (Ameye *et al*., 2018). In our case, it is likely that PAO resulted in these compounds bring protected from further oxidation and esterification, especially in the 2020 grapes. The total concentrations of phenols and vanillin-based compounds in the two wines were similar, the highest concentration being obtained for *p*-tyrosol, with no significant difference between the two wines, followed by acetovanillone, which was significantly higher in the control wine, and then 3,4,5-trimethoxy-phenol, with the highest concentration in the 2020 PAO wine (Table 3).

the total content of the bound form of the monoterpenes was about 30 % higher than that of the free form, and it was slightly higher in the control wine (Table 4) due to the greater amount (15-20 %) of several oxidised compounds, such as oxides, and hydroxylated forms, such as diols, which are markers of an oxidation process. In grapes, glycoside precursors are fairly abundant, especially in aromatic varieties, and bound glycosides forms are more abundant than the free ones (Mateo and Jimenez, 2000).

The identified diols were 2,6-dimetil-3,7-octadien-2,6-diolo and 3,7-dimetil-1,7-octadien-3,6-diolo (Rapp and Knipser, 1979; Rapp *et al*., 1980), which are characteristic of Muscat grapes with high bound fractions of terpenes. The concentrations of the bound form of exo-2-hydroxy-1,8-cineole - which was significantly high in the free form - was very low; meanwhile, of the bound monoterpenes, *trans*-8-hydroxylinalool showed the highest concentrations. Bound geraniol and nerol concentrations were significantly higher in the PAO wine than in the control wines. The concentrations of the bound C_{13}-norisoprenoids was almost 10 times higher than those of the free forms (which is usually the case (Baumes *et al*., 2002), with concentrations of vomifoliol (responsible for fruity aroma) almost 10-fold those of the other compounds and significantly higher in the PAO wines in the 2021 season. 2,3-dehydro-4-oxo-7,8-dihydro-β-ionone was also present in high concentrations regardless of the wines, and 3-oxo-α-damascone was significantly higher in the PAO wine. The total concentration of C_{13}-norisoprenoids was slightly higher in the PAO samples (1454 vs 1376 µg/L), but not significantly different. As C_{13}-norisoprenoids are derived from carotenoids, it follows that the abundance of C_{13}-norisoprenoids can be affected by the carotenoid profile of berries. In Nerello, the higher content of C_{13}-norisoprenoids compared with terpenes could be related to the genetic provenience from Sangiovese, which is known to be characterised as having high concentration of C_{13}-norisoprenoids (D'Onofrio *et al*., 2021). The higher concentration in PAO wines of some specific C_{13}-norisoprenoids could be due to the protection against oxidation occurring in vinification.

The content of bound C_6 was 6 to 8 times lower than that of the free C_6 and no differences were found between the two wines (Table 4). The concentrations of bound phenols and vanillin-derived compounds were approximately 1/7 those of the corresponding free compounds; after *p*-tyrosol, homovanillic acid was present in the highest concentrations, with significantly different values between the two wines, (118 vs 84 mg/L in PAO and Control respectively). The concentrations of Zingerone and *cis*-coniferol were also significantly higher in the PAO wine than in the control wine. The total concentrations of bound phenols and vanillin-derived compounds were higher in the PAO samples in both seasons (Table 4).

The free VOCs that were mainly derived from fermentation comprised, esters and alcohols. The total content of esters (Table 5) was significantly different between the two wines in the 2020 season only. A In terms of specific compounds,

TABLE 3. Free VOCs (µg/L) mainly derived from grapes in the PAO or control wines in the 2020 and 2021 seasons. Data are the mean (± SD) of three bottles. The values followed by different letters (within rows) are significantly different ($P < 0.05$).

	FREE VOCs	PAO 2020	Control 2020	PAO 2021	Control 2021
TERPENES	Linalool	3.91 ± 0.32	4.09 ± 0.99	3.61 ± 0.19	3.47 ± 0.78
	4-Terpineol	3.88 ± 0.33	3.96 ± 0.52	3.75 ± 0.19	3.66 ± 0.63
	α-Terpineol	1.67 ± 0.25	1.63 ± 0.23	1.37 ± 0.10	1.76 ± 0.23
	Citrale	1.09 ± 0.10	1.33 ± 0.11	1.17 ± 0.05	1.20 ± 0.11
	Oxide C (epoxylinalool)	1.94 ± 0.20	2.07 ± 0.19	1.77 ± 0.25	1.87 ± 0.17
	Oxide D (epoxylinalool)	1.59 ± 0.11	1.71 ± 0.11	1.67 ± 0.15	1.81 ± 0.10
	Citronellol	6.31 ± 0.32^{bc}	5.01 ± 0.53^{d}	7.01 ± 0.38^{ab}	6.00 ± 0.43^{c}
	Nerol	15.33 ± 0.22^{b}	14.22 ± 0.25^{c}	16.01 ± 0.42^{a}	15.34 ± 0.55^{b}
	p-Cymen-8-ol	5.28 ± 0.81	5.27 ± 0.24	4.71 ± 0.30	5.00 ± 0.44
	Geraniol	15.52 ± 0.68^{a}	14.11 ± 1.05^{b}	16.01 ± 0.32^{ab}	14.18 ± 0.79^{b}
	Exo-2-hydroxy-1,8-cineole	23.40 ± 2.20^{b}	77.92 ± 6.56^{a}	25.01 ± 0.81^{b}	87.92 ± 7.18^{a}
	Enediol	1.36 ± 0.11	1.47 ± 0.60	2.09 ± 0.38	1.47 ± 0.70
	trans-8-Hydroxylinalool	4.44 ± 0.04	4.36 ± 0.70	5.07 ± 0.70	4.66 ± 0.43
	Geranic acid	14.82 ± 0.81^{a}	9.07 ± 1.14^{b}	15.97 ± 0.68^{a}	8.01 ± 1.71^{b}
	Σ	101.33	147.24	104.79	157.77
C_{13}-NORISOPRENOIDS	Damascenone	2.76 ± 0.20	2.63 ± 0.80	3.12 ± 0.30	2.93 ± 0.67
	3-Hydroxy-ß-damascone	7.20± 0.52	6.42 ± 0.77	6.65± 0.58	6.30 ± 0.37
	3-Hydroxy-7,8-dihydro-ß-ionol	4.89 ± 0.93	4.85 ± 0.33	4.69 ± 0.80	3.99 ± 0.36
	3-Oxo-α-ionol	132.48 ± 11.46^{bc}	123.78 ± 15.81^{c}	145.40 ± 10.60^{ab}	120.88 ± 19.09^{c}
	Vomifoliol	45.32 ± 0.47^{a}	44.49 ± 2.90^{a}	37.84 ± 0.76^{b}	37.40 ± 3.91^{b}
	Actinidol A	2.34 ± 0.17^{b}	2.24 ± 0.10^{b}	3.01 ± 0.18^{a}	2.14 ± 0.10^{b}
	Actinidol B	4.01 ± 0.63	3.79 ± 0.10	4.19 ± 0.73	3.99 ± 0.19
	Σ	199.00	188.2	204.90	177.33
C6 COMPOUNDS	1-Hexanol	545.88 ± 37.12^{a}	474.22 ± 38.24^{b}	472.39 ± 45.08^{b}	428.38 ± 38.24^{b}
	trans-3-Hexen-1-ol	7.03 ± 0.42^{a}	6.69 ± 0.12^{b}	6.03 ± 0.32^{c}	6.00 ± 0.32^{c}
	cis-3-Hexen-1-ol	5.02 ± 0.33^{b}	7.91 ± 0.66^{a}	5.55 ± 0.26^{b}	7.99 ± 0.60^{a}
	trans-2-Hexenol	2.67 ± 0.46^{bc}	2.99 ± 0.13^{ab}	2.07 ± 0.40^{d}	2.18 ± 0.33^{cd}
	Σ	560.60	491.81	486.04	444.55
PHENOLS & VANILLIN-DERIVED COMPOUNDS	Phenol	4.89 ± 0.10	4.20 ± 0.42	4.97 ± 0.42	4.20 ± 0.27
	4-Ethylguaiacol	1.78 ± 0.15	1.88 ± 0.11	1.70± 0.15	1.88 ± 0.19
	4-Vinylguaiacol	44.3 ± 3.85	38.97 ± 4.32	38.87 ± 2.80	35.07 ± 4.09
	2,6-Dimethoxy-phenol	22.5 ± 1.33	21.67 ± 21.09	19.26 ± 1.99	19.98 ± 2.09
	Methoxyeugenol	6.66 ± 1.16	6.02 ± 0.94	6.09± 0.87	6.65 ± 0.44
	4-Ethoxymethyl-phenol	2.30 ± 0.63	2.18 ± 0.25	2.38 ± 0.43	2.18 ± 0.37
	Vanillin	3.59 ± 0.58	3.17 ± 0.33	3.87 ± 0.50	3.07 ± 0.30
	Homovanillyl alcohol	39.45 ± 4.22	44.06 ± 4.26	39.05 ± 2.98	39.29 ± 3.98
	Acetovanillone	177.64 ± 13.32^{bc}	204.59 ± 12.77^{a}	160.94 ± 18.97^{c}	198.66 ± 13.02^{a}
	Homosyringic acid	27.29 ± 2.15	23.59 ± 1.93^{b}	22.22 ± 2.10^{b}	22.59 ± 1.33
	Zingerone	4.63 ± 0.22^{c}	7.28 ± 0.74^{ab}	4.00 ± 0.22^{c}	6.88 ± 0.44^{b}
	3,4,5-Trimethoxy-phenol	39.13 ± 1.95^{a}	35.55 ± 2.52^{ab}	32.13 ± 0.86^{b}	32.87 ± 1.99^{b}
	p-Tyrosol	1628.41 ± 110.53	1654.24 ± 132.72	1690.00 ± 100.59	1711.28 ± 152.70
	Homovanillic acid	38.27 ± 4.13	32.57 ± 1.10	38.47 ± 3.70	38.50 ± 1.00
	Guaiacol	14.43 ± 1.00	15.24 ± 1.00	12.49 ± 1.07	15.00 ± 1.23
	Σ	2056.24	2095.21	2074.44	2135.70

TABLE 4. Bound VOCs (µg/L) mainly derived from grapes in PAO or control wines in the 2020 and 2021 seasons. Data are the mean (± SD) of three bottles. Values followed by different letters (within rows) are significantly different ($P < 0.05$).

Part 1/2

	BOUND VOCs	PAO 2020	Control 2020	PAO 2021	Control 2021
MONOTERPENES	Oxide A (epoxylinalool)	7.69 ± 0.96	7.71 ± 0.54	6.69 ± 0.60	7.33 ± 0.52
	Oxide B (epoxylinalool)	17.73 ± 1.22^{a}	18.51 ± 1.00^{a}	15.70 ± 1.54^{b}	18.00 ± 1.33^{a}
	Linalool	0.79 ± 0.11	0.76 ± 0.10	0.79 ± 0.18	0.76 ± 0.20
	α-Terpineol	5.37 ± 0.42^{ab}	4.86 ± 0.44^{bc}	5.57 ± 0.55^{ab}	4.00 ± 0.49^{c}
	Oxide C (epoxylinalool)	8.08 ± 0.72^{bc}	9.63 ± 0.44^{a}	7.78 ± 0.50^{c}	9.00 ± 0.55^{ab}
	Oxide D (epoxylinalool)	10.05 ± 0.27^{bc}	12.61 ± 0.55^{a}	9.46 ± 0.55^{c}	13.02 ± 0.65^{a}
	Citronellol	0.74 ± 0.22	0.83 ± 0.12	0.69 ± 0.20	0.80 ± 0.12
	Nerol	4.11 ± 0.35^{b}	3.28 ± 0.22^{c}	5.28 ± 0.29^{a}	3.68 ± 0.20^{c}
	Isogeraniol	0.69 ± 0.20^{c}	0.87 ± 0.13^{ab}	0.69 ± 0.29^{c}	0.77 ± 0.19^{bc}
	Lilac alcohol B	0.54 ± 0.10	0.71 ± 0.15	0.50 ± 0.10	0.71 ± 0.19
	Geraniol	22.20 ± 1.42^{a}	17.5 ± 1.16^{b}	22.87 ± 1.12^{a}	15.50 ± 1.36^{b}
	Exo-2-hydroxy-1,8-cineole	2.28 ± 0.33	2.17 ± 0.33	2.00 ± 0.33	2.00 ± 0.30
	Diol 1	5.75 ± 0.88	5.97 ± 0.86	5.95 ± 0.77	6.33 ± 0.66
	Enediol	2.54 ± 0.42	2.75 ± 0.57	2.14 ± 0.62	2.05 ± 0.88
	Diol 2	0.93 ± 0.12	1.23 ± 0.17	0.93 ± 0.12	1.23 ± 0.12
	Hydroxycitronellol	5.68 ± 1.01	6.07 ± 0.93	5.98 ± 1.31	6.12 ± 0.65
	trans-8-Hydroxylinalool	117.33 ± 10.92^{d}	128.58 ± 10.27^{cd}	132.59 ± 11.02^{bc}	137.30 ± 10.00^{ab}
	7-Hydroxygeraniol	6.76 ± 0.82^{cd}	7.96 ± 0.68^{ab}	5.99 ± 0.92^{d}	7.33 ± 0.38^{bc}
	cis-8-Hydroxylinalool	17.25 ± 1.21	18.38 ± 1.18	19.25 ± 1.81	16.38 ±0.99
	Geranic acid	10.70 ± 0.62^{b}	8.80 ± 0.51^{c}	11.70 ± 0.55^{a}	8.80 ± 0.22^{c}
	7-Hydroxyterpineol	11.61 ± 0.91	11.71 ± 1.41	11.77 ± 0.91	11.91 ± 1.09
	Σ	269.73	281.16	274.32	273.02
C_{13}-NORISOPRENOIDS	Actinidol A	3.10 ± 0.22	3.38 ± 0.24	2.99 ± 0.22	3.28 ± 0.54
	Actinidol B	5.52 ± 0.33	6.11 ± 0.73	5.77 ± 0.39	5.88± 0.73
	2,5,5,8 α-Tetramethyl-1,2,3,5,6,7,8,8 α -octahydro naphtalen-1-ol	2.11 ± 0.33	2.09 ± 0.13	2.00 ± 0.30	2.09 ± 0.43
	3,4-Dihydro-3-oxo-α-ionol (I)	42.78 ± 3.24	40.21 ± 3.24	40.88±3.00	42.99 ± 2.24
	3,4-Dihydro-3-oxo-α-ionol (II)	45.83 ± 2.73	43.87 ± 3.36	48.34 ± 1.99	45.02 ± 3.09
	3,4-Dihydro-3-oxo-α-ionol (III)	54.87 ± 2.95	52.84 ± 3.28	57.12 ± 3.11	50.84 ±2.99
	3-Oxo-α-damascone	23.10 ± 1.85^{a}	17.89 ± 1.39^{c}	20.19 ± 1.05^{b}	16.89 ± 1.40^{c}
	2,3-Dehydro-4-oxo-7,8-dihydro-ß-ionone	429.68 ± 39.26	445.73 ± 32.19	409.60 ± 28.97	405.23 ±37.10
	3,9-Dihydroxy-megastigma-5-ene	15.26 ± 1.53	14.53 ± 1.83	15.00 ± 1.67	12.53 ± 2.03
	Blumenol C	3.63 ± 0.53	4.03 ± 0.77	3.33 ± 0.53	4.00 ± 0.70
	Vomifoliol	738.95 ±47.20^{bc}	668.3 ± 54.14^{cd}	798.95 ± 42.29^{a}	630.33 ± 38.10^{d}
	7,8 Dihydrovomifoliol	29.95 ± 2.27^{b}	24.39 ± 1.94^{cd}	32.95 ±2.80^{ab}	20.39 ± 2.04^{d}
	Abscisic acid	59.48 ± 4.53	53.4 ± 3.24	59.00 ± 3.09	56.40± 2.24
	Σ	1456.26	1376.77	1496.12	1295.87
C6 COMPOUNDS	1-Hexanol	54.69 ± 2.63	54.31 ± 4.20	58.60 ± 3.13	54.00± 4.00
	trans-3-Hexen-1-ol	0.47 ± 0.10^{ab}	0.46 ± 0.16^{ab}	0.27 ± 0.30^{c}	0.36 ± 0.26^{bc}
	cis-3-Hexen-1-ol	5.80 ± 0.80	5.65 ± 0.17	5.80± 0.27	4.99 ± 0.39
	trans-2-Hexenol	19.74 ± 2.18	19.93 ± 0.76	18.64 ± 1.87	20.83 ± 0.67
	Σ	80.70	80.35	83.31	80.18

Part 2/2

	BOUND VOCs	PAO 2020	Control 2020	PAO 2021	Control 2021
PHENOLS & VANILLIN-DERIVED COMPOUNDS	Guaiacol	3.20 ± 0.43	3.62 ± 0.31	3.20 ± 0.78	3.12 ± 0.31
	p-Tyrosol	134.51 ± 10.97	137.20 ± 13.23	139.51 ± 10.0	130.20 ± 10.20
	2,6-Dimethoxyphenol	8.80 ± 0.39^{b}	11.87 ± 0.90^{a}	7.97 ± 0.99^{b}	11.92 ± 0.30^{a}
	Eugenol	13.55 ± 1.08^{d}	16.36 ± 1.77^{bc}	15.78 ± 1.32^{cd}	18.39 ± 1.70^{ab}
	Hydroxyisoeugenol	9.89 ± 0.95	8.19 ± 1.25	9.29 ± 0.90	8.19 ± 1.00
	3,4,5-Trimethoxyphenol	15.27 ± 0.84	14.54 ± 1.49	15.27 ± 1.12	13.54 ± 1.09
	cis-Coniferol 1	3.85 ± 0.43	3.87 ± 0.35	3.35 ± 0.54	3.47 ± 0.39
	cis-Coniferol	8.28 ± 0.43^{a}	6.56 ± 0.45^{b}	8.00 ± 0.23^{a}	6.09± 0.50^{b}
	Methyl vanillate	15.58 ± 1.10	16.18 ± 1.60	14.68 ± 1.00	15.78 ± 0.60
	Acetovanillone	4.93 ± 0.32	5.11 ± 0.77	4.43 ± 0.40	5.10 ± 0.67
	Zingerone	5.32 ± 0.43^{a}	3.98 ± 0.33^{b}	5.82 ± 0.66^{a}	3.55 ± 0.53^{b}
	Homovanillic alcohol	31.50 ± 1.45	29.28 ± 1.42	29.89 ± 2.09	32.28 ± 2.40
	Methyl syringate	4.65 ± 0.65	4.97 ± 0.21	4.05 ± 0.65	4.97 ± 0.32
	Homovanillic acid	118.00 ± 8.77^{a}	83.88 ± 5.53^{b}	128.05 ± 10.07^{a}	83.98 ± 3.53^{b}
	Acetosyringone	25.19 ± 1.18^{a}	22.38 ± 1.24^{b}	20.19 ± 1.20^{c}	24.38 ± 1.08^{a}
	Σ	402.52	367.99	409.43	364.96

the highest values were obtained for ethyl acetate in the PAO samples in both seasons, followed by ethyl hydrogen succinate (no significant differences), and diethyl succinate (significantly higher in the control wine).

In the 2020 season, the PAO wine was characterised as having higher concentrations of different esters than the control wines, such as isoamyl acetate, isopentyl acetate, ethyl crotonate, ethyl hexanoate, ethyl lactate, ethyl-2-furoate and diethyl malate; however, they were all present in low concentrations. Ethyl fatty acid esters are compounds primarily important for their contribution to wine aroma. Ethyl lactate, one of the most characteristic aromatic compounds to be released during malolactic fermentation, is synthesised during the course of the esterification of ethanol (produced by yeast during alcoholic fermentation) and lactate (produced by malolactic bacteria during malolactic fermentation). During the malolactic process, the concentration of ethyl lactate progressively increases, contributing to wine aroma with fruity, buttery and creamy notes, as well as to the organoleptic sensations of roundness in the mouth (Ugliano and Moio, 2005). Diethyl succinate is derived from succinic acid (a by-product of microbial α-ketoglutarate metabolism) during fermentation, and it contributes to aroma with fruity melon notes; its odour threshold has been set at 1.2 µg/L (Peinado *et al*., 2004). In our wines, the concentrations of diethyl succinate were much higher: 5.4 and 4.0 µg/L in the control and PAO wines respectively. Ethyl acetate can be formed as a result of chemical or biochemical reactions, and, at concentrations of below 100000 µg/L, as in our case, it has a desirable fruity aroma, thus improving wine quality. Isoamyl acetate has ripe banana notes, and is formed from isoamyl alcohol and acetic acid, which are intermediate metabolites of alcoholic and malolactic fermentation. Here, the measured concentrations were higher in PAO wine than in the control wine. The significantly higher concentrations of acetate esters in PAO wine were unexpected, because the low oxygen contact should have prevented any oxidation. It appears that in PAO vinification, a higher concentration of esters from fatty acids been measured thus it is possible that higher concentration of acetyl-CoA will be available. In anaerobic microorganisms, the Krebs cycle has been found to invert its cycle, provoking the formation of high concentrations of acetyl-CoA (Steffens *et al*., 2021).

Alongside esters, alcohols are important aromatic compounds produced during fermentation. Of the free alcohols, 2-phenylethanol was present in the highestconcentrations (more than 13000 µg/L in both wines) with no significant differences, followed by isoamyl alcohol (6622 and 7707 µg/L respectively) in the control and PAO wines (Table 5). No differences were found in terms of the other alcohols between the two wines in both seasons, except for *trans*-hexenol which was significantly higher in the control wines.

As regards the bound volatile compounds mainly derived from fermentation, no esters were detected and very low levels of alcohol were measured without significant differences (data not shown).

Finally, regarding thiols in just the first season (Table 6), no 3-sulfanyl-hexyl acetate (3SHA) was detected, while the concentrations of 3-sulfanylhexan-1-ol (3SH) were higher than the odour threshold cited in the literature (60 ng/L, Tominaga *et al*., 1998), being significantly higher in the PAO wine.

3-sulfanylhexan-1-ol gives wine a grapefruit and passion fruit aroma. This is the first time that thiols have been measured in Nerello Mascalese wine.

TABLE 5. Free VOCs (µg/L) mainly derived from fermentation in PAO or control wines in the 2020 and 2021 seasons. Data are the mean (± SD) of three bottles. Values followed by different letters (within rows) are significantly different ($P < 0.05$).

Part 1/2

		PAO 2020	Control 2020	PAO 2021	Control 2021
ESTERS	Ethyl acetate	76655.41 ± 3666.20^{ab}	67448.44 ± 2450.10^{cd}	70234.11 ± 3266.28^{bc}	64428.44 ± 2098.10^{d}
	Isoamyl acetate	2230.90 ± 190.71^{a}	1734.57 ± 195.10^{b}	2100.90 ± 100.01^{a}	1700.50 ± 105.00^{b}
	Isopentyl acetate	532.42 ± 44.22^{a}	364.98 ± 56.20^{b}	560.22 ± 34.20^{a}	329.18 ± 42.20^{b}
	Ethyl crotonate	3.20± 0.70^{a}	0.20± 0.00^{b}	3.00± 0.30^{a}	0.01± 0.00^{b}
	Ethyl hexanoate	145.12 ± 13.26^{a}	101.70 ± 22.75^{cd}	115.12 ± 10.29^{bc}	93.72± 10.02^{d}
	Hexyl acetate	2.20 ± 0.24	2.33 ± 0.82	2.20 ± 0.32	2.03 ± 0.12
	Ethyl (S)-(-)-lactate	1280.86 ± 110.12^{a}	1043.07 ± 100.15^{b}	1295.80 ± 90.18^{a}	1072.07 ± 121.10^{b}
	Ethyl octanoate	263.23 ± 18.23^{b}	235.85 ± 18.20^{cd}	278.29± 20.23^{ab}	200.81 ± 13.00^{d}
	Ethyl 3-hydroxybutanoate	19.88 ± 1.23^{a}	14.23 ± 1.12^{b}	20.11 ± 0.98^{a}	14.00 ± 1.09^{b}
	Ethyl 2-hydroxy-4-methylpentanoate	111.93 ± 11.17^{bc}	97.72 ± 8.20^{cd}	120.92 ± 10.00^{ab}	92.09 ± 8.01^{d}
	Isoamyl lactate	75.38 ± 6.54	76.29 ± 8.55	78.72 ± 6.00	69.23 ± 4.99
	Ethyl 2-furoate	5.40 ± 0.99^{a}	2.87 ± 0.20^{b}	5.29 ± 0.72^{a}	2.56 ± 0.32^{b}
	1-Ethyl 4-methyl succinate	22.24 ± 0.25^{b}	27.15 ± 3.45^{a}	22.14 ± 0.23^{b}	20.23 ± 2.99^{b}
	Ethyldecanoate	97.98 ± 0.95	90.78 ± 8.20	104.20 ± 3.78	94.26 ± 5.53
	Diethyl succinate	4005.33 ± 364.00^{b}	5433.22 ± 236.60^{a}	4125.33 ± 210.00^{b}	5632.19 ± 210.98^{a}
	1,3-Propanediol, diacetate	35.50 ± 1.95	41.91 ± 3.90	38.00 ± 1.05	40.91 ± 1.90
	Diethyl 2-methylenesuccinate	4.19 ± 0.74	4.06 ± 0.75	3.74 ± 0.66	3.86 ± 0.29
	Benzeneacetic acid, ethyl ester	4.52 ± 0.63	4.64 ± 0.70	4.02 ± 0.34	4.78 ± 0.20
	Succinic acid, butyl ethyl ester	10.21 ± 1.09	12.47 ± 1.60	10.11 ± 1.00	12.28 ± 1.00
	ß-Phenethyl acetate	162.62 ± 10.13^{b}	199.27 ± 18.30^{a}	157.22 ± 10.34^{b}	190.00 ± 12.22^{a}
	Ethyl 2-(hydroxymethyl)butanoate	76.82 ± 4.00	87.60 ± 6.10	78.82 ± 3.09	80.62 ± 4.20
	Diethyl malate	112.50 ± 9.93^{a}	88.58 ± 6.70^{b}	100.50 ± 9.02^{a}	77.11 ± 6.99^{b}
	Ethyl 3-hydroxytridecanoate	5.58 ± 0.54	5.84 ± 0.90	5.08 ± 0.14	4.84 ± 0.33
	Succinoic acid, 2-hydroxy-3-Methyl-, diethyl ester	523.74 ± 43.28	477.74 ± 27.60	498.11.74 ± 37.22	435.23 ± 18.22
	Ethyl-5-oxy-4H-2-furancarboxylate	1539.18 ± 109.27	1401.97 ± 104.90	1487.12 ± 134.29	1487.10 ± 108.00
	Ethyl hydrogen succinate	9766.11 ± 543.29	10000.10 ± 1080.91	9320.00 ± 345.29	10120.98 ± 992.02
	Methyl vanillate	18.20 ± 1.19	18.24 ± 1.20	18.20 ± 1.00	17.24 ± 0.90
	Ethyl vanillate	179.60 ± 11.50	204.73 ± 18.62	119.60 ± 11.50	229.73 ± 11.36
	Methyl gentisate	24.02 ± 0.92	23.04 ± 1.95	23.20 ± 0.92	23.98 ± 1.05
	Ferulic acid, ethyl ester	11.72 ± 1.00	10.18 ± 2.10	10.72 ± 0.56	12.87 ± 1.23
	Ethyl-β-(4-hydroxy-3-methoxy-phenyl)-propionate	10.24 ± 1.10	9.19 ± 1.80	10.00 ± 0.87	11.00 ± 1.20
	Σ	97935.68	89262.76	90949.89	86506.85

		PAO 2020	Control 2020	PAO 2021	Control 2021
ALCOHOLS	Isoamyl alcohol	7707.43 ± 543.10^{a}	6622.85 ± 580.10^{b}	7802.43 ± 343.20^{a}	6555.08 ±398.02^{b}
	4-Methyl-1-pentanol,	16.63 ± 2.70	13.19 ± 0.20	19.90 ± 1.99	13.78 ± 0.29
	3-Methyl-2-buten-1-ol,	6.01 ± 0.80	6.26 ± 0.20	6.00 ± 0.35	6.00 ± 0.20
	3-Methyl-1-pentanol	21.08 ± 20^{bc}	18.24 ± 2.10^{c}	26.20 ±1.00^{a}	19.24 ± 2.00^{c}
	1-Hexanol	545.88 ± 37.10^{a}	474.22 ± 38.20^{b}	507.88 ± 39.00^{ab}	437.11 ± 28.20^{b}
	trans-3-Hexen-1-ol	5.25 ± 0.90	4.86 ± 0.10	5.89 ± 0.60	4.80 ± 0.18
	3-Ethoxy-1-propanol	6.09 ± 0.90	6.81 ± 1.00	6.10 ± 0.10	6.11 ± 122
	3-Hexen-1-ol	7.03 ± 0.70	6.69 ± 1.10	7.77 ± 0.38	6.79 ± 1.21
	trans-2-Hexenol	5.02 ± 0.90^{c}	7.91 ± 0.60^{a}	5.29 ± 0.32^{c}	7.11 ± 0.64^{a}
	cis-2-Hexen-1-ol	2.67 ± 0.40	2.99 ± 0.70	2.00 ± 0.32	1.99 ± 0.73
	Ethyl hexanol	3.30 ± 0.40	3.49 ± 0.40	2.98 ± 0.40	2.99 ± 0.53
	Octanol	7.85 ± 0.20	6.68 ± 0.60	7.07 ± 0.32	6.98 ± 0.45
	2,3,4-Trimethoxybenzyl alcohol	11.28 ± 1.10	10.17 ± 1.70	11.98 ± 1.01	11.00 ± 0.99
	1H-indole-1-ethanol	42.67 ± 2.30	47.9 ± 6.70	39.60 ± 1.30	44.98 ± 3.82
	Benzyl alcohol	81.45 ± 5.50	77.78 ± 6.70	79.15 ± 3.90	75.20 ± 3.20
	2-Phenylethanol	13747.60 ± 1009.10	13360.7 ± 860.20	13980 ± 999.87	13120.7 ± 670.20
	2-(4-Methoxyphenyl)-ethanol	15.65 ± 1.10	16.083 ± 2.20	14.65 ± 0.99	15.78 ± 1.08
	Σ	22232.89	20686.82	22522.89	20335.64

TABLE 6. Free thiols (ng/L) identified in wines from PAO (protection against oxygen) or traditional winery winemaking (Control); we performed this analysis only in season 2020. Data are the mean (± SD) of three bottles. The asterisk indicates a significant difference between the two samples ($P < 0.05$).

THIOLS	PAO 2020	Control 2020
4-Methyl-4-sulfanyl pentan-2-one	3.8 ± 0.5	4.1 ± 0.6
3-Sulfanyl hexan-1-ol	740.4 ± 65.0	588.0 ± 37.2*
Ethyl-2-sulfanyl acetate	454.3 ± 30.4	512.0 ± 36.7
Benzenemethanethiol	11.6 ± 1.7	11.4 ± 0.9
Σ	1210.1	1115.5

Ethyl-2-sulfanyl acetate (E2SA), an off-odour compound, was also detected in high concentrations (Table 6). This compound was first identified as having an off-odour of cooked beans in a number of Sauvignon blanc wines made from hard-pressed juices in an inert atmosphere (nitrogen) or in contact with oxygen (Nikolantonaki and Darriet, 2011). In dry white wine, the threshold for considering an odour as an off-odour was defined as being between 300 and 500 ng/L. Dissolved oxygen in must modulates the metabolic activity of yeasts, promoting E2SA formation from its low molecular weight precursor; this would explain the slightly higher concentration in the control wine. Potent varietal thiols or sulfanyl compounds are major contributers to the typical aroma of Sauvignon blanc wines, as well as wines made from many other white and red grape varieties (Chenin blanc, Gewürztraminer, Semillon, Petit Manseng, Arvine, Colombard, Merlot, Cabernet-Sauvignon), including dessert wines (Sarrazin *et al*., 2007); however, generally speaking, volatile compounds can also contribute to aroma attributes as a result of complex additive effects or synergistic and/or masking phenomena (Darriet *et al*., 2013; Ferreira *et al*., 2021). It should be noted that the Etna area is volcanic, and the underground water is classified as "sulfureous" due to it containing 45 mg/L of sulfur compounds, and as "mineral" due to it containing 7 g/L of salts (Terme Acireale, 2017). Etna volcano is still active; thus the air around the volcano where vines are grown is rich in sulfur compounds. In this macroclimate, the vines and grape clusters absorb sulfur compounds not only from the soil but also from the air (as is the case with smoke taint); these compounds may have been transformed within the berry cells into the odorant compounds we detected, thus explaining their high concentrations. Compounds like 3SHA, 3SH, E2SA or 4-MSP may have been produced by the berry cell during

TABLE 7. Olfactive and gustative main attributes (0 – 10) of PAO and control wines. Three bottles were tasted by 10 panelists in both years. Similar results were obtained in both years, thus data are the mean (± SD) of the two-year sensory analysis. The asterisk indicates a significative difference ($P < 0.05$).

ATTRIBUTE	CONTROL	PAO
Red fruit aroma	6.9 ± 0.6	5.6 ± 0.5*
Herb aroma	5.3 ± 0.4	2.8 ± 0.4*
Flowery aroma	4.3 ± 0.8	6.0 ± 0.6*
Mature pear, senescent cut flower aroma	3.6 ± 0.4	0*
Taste astringency	7.8 ± 0.8	6.6 ± 0.6
Mineral taste	4.0 ± 0.3	5.0 ± 0.5*
Taste persistence	8.2 ± 0.9	9.0 ± 0.9
Taste intensity	7.8 ± 0.6	9.1 ± 0.7*

ripening, since we know that during prolonged ripening the berry can become enriched with ethanol and acetic acid, as well as with hexanol, which we found in high concentration.

4-methyl-4-sulfanyl pentan-2-one (4-MSP) (associated with aromatic nuances of blackcurrant, passion fruit, box tree and broom) was found in low concentrations, which were nonetheless higher than the odour threshold (3.0 ng/L; (Darriet *et al*., 1995; Tominaga *et al*. 1998). Finally, benzenemethanethiol is a volatile thiol with a strong empyreumatic aroma and a very low odour threshold (0.3 ng/L; Tominaga *et al*., 2003); in our case, the value was much higher (more than 11 ng/L) without any significant differences between the two wines.

As regards the sensory analysis (Table 7), the assessors significantly perceived a red fruit and herb aroma characterizing the Control wine which can be attributed to the high concentration of exo-2-hydroxy-1,8-cineole. Overall, assessors preferred the PAO winefor its flowery aroma (nerol, linalool, geraniol), mineral taste and the taste intensity (higher acidity and polyphenol content).

An oxidative nuance in the control wine identified as mature pear or senescent cut flower was perceived by all the panelists.

CONCLUSIONS

The main differences between the two otherwise similar winemaking processes applied in this study were the aeration during maceration/fermentation in the pump-over and délestage and the steps taken to avoid contact with oxygen during racking. The production of Nerello Mascalese via the PAO winemaking process, altered some specific VOCs and thus influenced the aroma of the wine. PAO wine contained higher concentrations of terpenes and nor-isoprenoids in the bound form than in the free form; moreover C_{13}-norisoprenoids in particular were present in slightly higher concentrations in the PAO wines. Of the monoterpenes, the concentrations of exo-2-hydroxy-1,8-cineole were significantly higher in the control wine. This is the first time that thiols have been measured in Nerello. 3-sulfanylhexan-1-ol was detected in high concentrations in PAO wine, and high concentrations of ethyl-2-sulfanyl acetate were present in both wines (higher in PAO wine). Related to fermentation, VOCs alcohols, such as 2-phenylethanol, isoamyl alcohol and benzyl alcohol were detected in higher concentrations in PAO wines, whereas the total ester content was significantly different between the two wines in the 2020 season only.

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