ORIGINAL RESEARCH ARTICLE

Identifying Wine Grape Aromatic Maturity using E-nose and GC-MS: the case of Nerello Mascalese Grapes from two Contrade of the Etna Area

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ABSTRACT

A study of aromatic maturity of Nerello Mascalese grapes from two districts ("Contrade") of the Etna area was carried out using gas chromatography-mass spectrometry (GC-MS) and electronic nose (E-nose). To validate our hypothesis regarding the potential use of E-nose for aromatic maturity, two vineyards with different characteristics (08 Alto and Solicchiata) were used. Grapes were sampled at 18°, 21° and 23°Brix. Regarding the phenol maturity index, total anthocyanins reached a peak at the second sampling in 08 Alto grapes, while in Solicchiata they constantly increased. The ratio total anthocyanins: total polyphenols in 08 Alto grapes increased from 0.14 to 0.33, and in Solicchiata from 0.17 to 0.23. As regards grape volatile organic compounds (GVOCs) for the aromatic maturity index, in Solicchiata the concentrations of glycosylated benzenoids and C_{13}-norisoprenoids were much higher than in 08 Alto, and the concentration decreased during maturity (opposite trend to the anthocyanins); by contrast, in 08 Alto, concentrations peaked at the second sampling time (as with the anthocyanins). The E-nose results did not completely coincide with the GVOCs pattern, but they discriminated the maturity stages very well. However, the different metallo-porphyrins responded differently depending on the class of GVOCs, highlighting very promising results in terms of GVOCs non-destructive prediction by means of principal component regression (PCR) application. E-nose shows potential for easy use with rapid PCR for the monitoring of the aromatic maturity of Nerello Mascalese grapes.

INTRODUCTION

The decision regarding the time of wine grape harvest is typically made by the grower or winemaker by taking several factors into account, such as grape phenology, harvest and processing logistics, and the forecasted weather conditions (Menzel *et al.,* 2006). The metric 'day of year maturity' (DOYM) has been used by several researchers (Jarvis *et al.,* 2017; Petrie and Sadras, 2008; Webb *et al.,* 2011). DOYM describes the date (or day of year) at which wine grapes reach a desired sugar concentration, which can differ between grape varieties or within the same grape variety in different regions (Jarvis *et al.,* 2017). In the present study, the word 'maturity' in this context is used to describe this chosen metric and does not imply that the grapes are mature as such.

Wine grape maturity can be classified according to four different types: technological, phenolic, cellular and aromatic. While technological maturity is easy to analytically determine (by analysing sugar and acidity levels), the evaluation of phenolic maturity is more complex, as is, to an even greater extent, the evaluation of cellular and aromatic maturity. For the consumer, the wine aroma is the main feature of judgement, thus aromatic maturity is the most important of the aforementioned maturity types. Unfortunately, as already mentioned, its identification is very challenging and requires expensive and complex laboratory instruments. In this context, the use of electronic nose (E-nose) has been proposed as reliable tool to monitor the aromatic maturity of different wine grape varieties (Aleixandre *et al.,* 2015; Athamneh *et al.,* 2008). Furthermore, near-infrared spectroscopy (NIRS) has been widely used for grape and wine analysis (Cozzolino *et al.,* 2006) and specifically to monitor technological (Barnaba *et al.,* 2014; Fernández-Novales *et al.,* 2009; Kalopesa *et al.,* 2023; Ribera-Fonseca *et al.,* 2016) and aromatic maturity (Boido *et al.,* 2013; Gehlken *et al.,* 2023), as well as phenolic composition (Cozzolino *et al.,* 2004; Rolle *et al.,* 2012).

Nerello Mascalese is a southern Italian red grape variety which has found optimal growing conditions in the Etna Volcano area. Nerello Mascalese, genetically speaking, originates from Sangiovese and Mantonico bianco varieties (D'Onofrio *et al.,* 2021). The wines are characterised by low polyphenol content (1945 – 2000 mg/L) and anthocyanin content (around 125 mg/L) (Giacosa *et al.,* 2023). Regarding volatile organic compounds (VOCs), Ansaldi *et al.* (2014) reported *trans*-8-hydroxy linalool and geraniol to be the most abundant terpenes, and 3-oxo-α-ionol and vomifoliol in the class of C_{13}-norisoprenoids. Despite its terpenic profile, Nerello Mascalese is not considered an aromatic variety. To date, no scientific papers have been published on the aromatic maturity of Nerello Mascalese and based on our knowledge on the application of E-nose (consisting of polymeric films of metal-porphyrins, deposited on quartz microbalances (QMB)), we hypothesised that E-nose and fast PCR is a rapid and useful tool for determining aromatic maturity. We therefore carried out an in-depth study to determine the potential of electronic nose (E-nose) for monitoring aromatic maturity and compare it with GC-MS (gas chromatography-mass spectrometry).

To this end, we chose two vineyards located in two districts ("Contrade") of the Etna area. These vineyards were 5 km from each other, but had completely different characteristics in terms of climate, one being close to a river and the other being at a higher elevation. To validate the use of E-nose in the study of aromatic maturity, the grapes from both vineyards were harvested at three developmental stages and at different sugar levels in degrees Brix (T0 = 18 ± 1 °Bx, T1 = 21 ± 1 °Bx and T2 = 23 ± 1 °Bx).

MATERIALS AND METHODS

1. Materials and experimental procedure

The experimental studies were carried out in two Nerello Mascalese (*Vitis Vinifera*) vineyards located in two districts

TABLE 1. Chemical-physical composition of the vineyard soils.

Parameters	Units	08 Alto	Solicchiata
Sand	%	70 ± 3	80 ± 2
Silt	%	25 ± 1	18 ± 1
Clay	%	5 ± 0	2 ± 0
Organic matter	%	1.1 ± 0.1	1.2 ± 0.1
Total limestone	%	< LOQ	< LOQ
Active limestone	%	< LOQ	< LOQ
Total nitrogen	g/kg	3.1 ± 0.1	4.3 ± 0.2
C/N	-	12.6 ± 0.3	14.4 ± 0.2
pH	-	7.63 ± 0.03	7.01 ± 0.02
Cation Exchange Capacity	meq/100 g	24.5 ± 0.3	22.0 ± 0.3
Magnesium	meq/100 g	3.1 ± 0.2	1.7 ± 0.1
Potassium	meq/100 g	1.0 ± 0.1	1.2 ± 0.1

Data are the mean (± standard deviation) of three soil samples. LOQ = Limit of Quantification.

("Contrade") 5 km from each other: 08 Alto and Solicchiata. The Etna area is known for its variable orographic and climatic conditions. The vineyards are located in the northern area of the Etna region. The 08 Alto vineyard was planted in 2008, has an area of 1.2 ha, an elevation of 555 m a.s.l. and a north-south orientation. Solicchiata vineyard contains older vines that were planted about 40 years before the time of writing, and has an area of 0.5 ha, an elevation of 678 m a.s.l. and a north-south orientation. The adopted pruning system is double spurred cordon with 10 buds per vine, a vine distance of 1.4 m x 2.3 m and the same clone grafted onto R108 rootstock. Both vineyards are managed in the same way. The chemical-physical composition (provided by the winery) of the vineyard soil is reported in Table 1: according to the typical characteristics of Etna area, the soil mostly consists of sand without limestone.

Both vineyards have similar soil characteristics, despite 08 Alto being close to a river and Solicchiata being far from the river with a different soil texture (less stony). The orographic situation and the presence of the river affects the microclimate. The 08 Alto vineyard environment is typically more humid, sometimes reaching dew point on the leaves, while Solicchiata has a windier climate with a light breeze in the afternoon.

In the experiment, grape maturity was monitored in each vineyard. Fifteen grape clusters were collected at different points (according to a cross design) in the vineyard and at three maturity stages based on sugar content (T0 = 18 ± 1 °Bx, T1 = 21 ± 1 °Bx and T2 = 23 ± 1 °Bx). The 15 grape bunches were then destemmed and divided into three lots (5 bunches each), each lot crushed using a juice extractor (JU3701 Frutelia Centrifuge Moulinex, Ecully, France). The resulting must immediately underwent titratable acidity analysis (g/L tartaric acid). The rest of must of each lot was frozen for further analyses.

2. Chemical analysis

The frozen must was thawed, centrifuged (6869 g for 5 min at 22 °C), filtered (0.82 μm paper filter) and analysed using a calibrated Fourier transform infrared WineScan® FT 120 (Foss Analytics, Hillerod, Denmark) to determine the following oenological parameters: pH, titratable acidity (g/L tartaric acid), volatile acidity (g/L acetic acid), malic acid (g/L), tartaric acid (g/L), lactic acid (g/L), glycerol (g/L), yeast assimilable nitrogen (YAN) (mg/L), SO_4 (g/L), total anthocyanins (mg/L malvidin), and total polyphenols (mg/L gallic acid). Each sample (each lot) was analysed three times; 3 WineScan® readings. The accuracy of the WineScan® analyses was verified via destructive analyses performed using the standard methods as previously reported (Bianchi et al., 2021).

3. HPLC analysis

Grape monoglucoside-anthocyanins were characterised by HPLC using a PU-2089 Plus quaternary pump (Jasco International Co., Ltd., Tokyo, Japan) equipped with a degasser, an AS-2057 Plus autosampler (Jasco International Co., Ltd., Tokyo, Japan) and a CO-2060 Plus column oven (Jasco International Co., Ltd., Tokyo, Japan). Detection was carried out using an UV-2070 Plus visible detector (Jasco International Co., Ltd., Tokyo, Japan). The data were processed by ChromNAV (software version 2.3). For the analytical determination of anthocyanins, a quantity of 1 mL of grape juice diluted 1:1 with phase A was taken from the samples. The sample obtained was filtered through a 0.45 μm PVDF (Polyvinylidene fluoride) filter and then injected into the HPLC. The separation was carried out with a DionexAcclaim®120 C18 column, 5μm, 4.6×250 mm thermostated at 30 °C. The mobile phase consisted of a ternary gradient: solvent A = 50 mM ammonium dihydrogen phosphate adjusted to pH 2.6 with acid phosphoric, solvent B = 20 % solvent A and 80 % acetonitrile, and solvent C = 0.2 M orthophosphoric acid adjusted to pH 1.5 with NaOH. The phenolic compounds were identified based on their elution order, the retention times of the pure compounds and the characteristics of their UV-Vis spectra at a wavelength of 520 nm for anthocyanins.

4. GC-MS analysis

GVOCs and their glycosylated precursors were determined as described by Corona et al. (2020) and García-Muñoz et al. (2011) with some modification. One hundred grams of must was centrifuged at 1968 g for 15 min. The liquid was collected in an Erlenmeyer flask and pectolytic enzyme without glycosidase activity was added to it (Vinozym, Novo Nordisk Ferment Ltd, Dittingen). The liquid was stored at 4 °C for 24 h. The pellet was resuspended in 50 mL of pH 3.2 tartaric buffer (5 g/L of tartaric acid brought to pH 3.2 with 22.2 mL of 1 M sodium hydroxide) and kept in contact with it for 24 hours. After 24 hours the grape juice was filtered through a paper filter (Whatman®, 589 mm), the tartaric buffer was centrifuged at 1968 g for 10 min and filtered through the same filter as the grape juice. Both liquids were combined and 0.5 mL of internal standard (1-heptanol 35.05 mg/L in 10 % v/v of ethanol) was added. The aroma compounds of the grape extract were concentrated through solid phase extraction (SPE) on 5 g ISOLUTE® C18 (Biotage, Uppsala, Sweden) previously activated with 25 mL of methanol and washed with 50 mL of distilled water. The free fraction was eluted with 25 mL of dichloromethane and the glycosylated fraction with 25 mL of methanol. The methanol of glycosylated fraction was evaporated through rotavapor (BÜCHI Labortechnik AG R-210, Flawill, Switzerland) at 40 °C and the dry extract was re-dissolved in 5 mL of citrate-phosphate buffer with a pH of 5, to which 200 μL of glycosidase enzymes were added (Cytolase M102, Ferrari srl, Verona, Italy). This glycosylated fraction was kept at 40 °C for 21 h to liberate the glycosylated aroma precursors. Subsequently the liberated fraction was concentrated through SPE with 1 g ISOLUTE® C18 (Biotage, Uppsala, Sweden) activated with 12 mL of methanol and washed with 24 mL of water. The elution was carried out using 12 mL of dichloromethane. All the dichloromethane extracts were dried with anhydrous sodium sulphate and concentrated at 500 μL. One μL was injected in splitless mode in a 6890 GC (Agilent, Santa Clara, USA) coupled with a 5973N mass

spectrometer (Agilent, Santa Clara, California). The GC was equipped with a DB-WAX column (Agilent Technologies; 30 m, 0.250 mm, 0.25 μm). The oven thermal programme was 40 °C for 2 min, 30 °C/min until 60 °C, 2 °C/min until 190 °C, 5 °C/min until 230 °C, and 230 °C for 15 min. The temperature of the transfer line was 230 °C. The m/z acquisition range was 30-350. The integration was done in total ion current mode expressing the concentration of the analytes in the equivalent of 1-heptanol of grape must.

5. E-nose analysis

The GC-MS analysis of the GVOCs was compared with the non-destructive measurement performed through E-nose using 8 sensors consisting of polymeric films of metal-porphyrins deposited on quartz microbalances (QMB). The E-nose used in this study had been designed, developed and assembled at the University of Rome Tor Vergata (Di Natale *et al.,* 2007). Each QMB was an electromechanical resonator whose frequency changes in proportion to the mass adsorbed on the sensor surface. Sensors were based on AT-cut quartz plates which oscillated at a resonance frequency of 20 MHz in the thickness shear mode (Di Natale *et al.,* 2007). The array was controlled by computer software, which received the sensor signals for subsequent analysis. The sampling protocol for the musts analysis was as follows. The frozen must was thawed and 5 mL of must was incubated at 20 ± 2 °C in a flask containing 5 mL of a saturated solution of $CaCl_2$ for 30 min. The equilibrated headspace was then extracted by means of a stream of filtered air and delivered into the E-nose. After measurement, a pure nitrogen stream was used to clean the sensors and establish the reference signal. The sensor signals were calculated as the shifts of the resonant frequency between the two steady conditions. Each sample was analysed in triplicate. Once the acquisitions were completed, the mean values were assembled in an overall matrix and mean-centered for subsequent multivariate statistical evaluation. For the construction of the principal component analysis model, leave-one-out was used as a validation method and three principal components were selected to reach a cumulative % of explained variability of about 95 %.

6. Statistical analysis

Statistical analysis was performed using SPSS version 17.0 for Windows (SPSS Inc., Chicago, IL, USA). The Tukey test for $p \leq 0.05$ was used in order to establish statistical differences by one-way analysis of variance (ANOVA).

All multivariate analyses were used: principal component analysis (PCA), hierarchical cluster analysis (HCA) and principal component regression (PCR) using Matlab R2021a (MathWorks®, Natick, MA, USA), PLS Toolbox 9.0 (Eigenvector Research, Inc., Manson, WA, USA), and Past 4.04 software (Hammer *et al.,* 2001).

RESULTS AND DISCUSSION

1. Oeno-chemical characterisation

Nerello grape harvested from the two vineyards showed significant difference in terms of main oeno-chemical parameters (i.e., the grape chemical characteristicsthat are important for the production of wine) during maturity (Table 2).

As expected, pH rose and titratable acidity decreased with similar values in both the 08 Alto and Solicchiata grapes. Tartaric acid decreased in both samples during maturity, but at a higher rate in the 08 Alto grapes. During maturity, volatile acidity increased in 08 Alto and decreased in Solicchiata. Malic and lactic acid decreased in both grapes, with the latter found at very low levels, as expected, compared with the other acids.

TABLE 2. Oeno-chemical parameters of juice from grapes of 08 Alto and Solicchiata vineyards.

Parameters	Units	08 Alto			Solicchiata		
		T0	T1	T2	T0	T1	T2
pH	-	3.16 ± 0.06 c	3.35 ± 0.01 b	3.50 ± 0.05 a	3.23 ± 0.01 c	3.51 ± 0.01 a	3.59 ± 0.06 a
Titratable acidity	g/L tartaric acid	9.21 ± 0.31 b	7.93 ± 0.21 c	6.78 ± 0.42 d	9.82 ± 0.11 a	7.69 ± 0.11 c	7.08 ± 0.05 d
Volatile acidity	g/L acetic acid	0.10 ± 0.02 c	0.29 ± 0.01 a	0.31 ± 0.013a	0.31 ± 0.04a	0.31 ± 0.014a	0.18 ± 0.01 b
Malic acid	g/L	3.14 ± 0.15 b	2.15 ± 0.10 d	2.41 ± 0.11 c	3.48 ± 0.11 a	2.50 ± 0.10 c	2.04 ± 0.17 d
Lactic acid	g/L	0.07 ± 0.02 b	n.d.	0.02 ± 0.00 c	0.25 ± 0.02 a	n.d.	0.03 ± 0.00 c
Tartaric acid	g/L	6.25 ± 0.20 a	4.69 ± 0.12 c	3.75 ± 0.14 e	5.77 ± 0.12 b	4.40 ± 0.22 d	4.50 ± 0.13 cd
Glycerol	g/L	n.d.	0.28 ± 0.03 c	0.38 ± 0.02 b	0.04 ± 0.01 d	0.29 ± 0.03 c	0.50 ± 0.03 a
YAN	mg/L	85 ± 6 e	145 ± 8 d	180 ± 5 c	177 ± 5 c	235 ± 6 a	202 ± 9 b
SO_4	g/L	0.62 ± 0.04 bc	0.65 ± 0.08 b	0.81 ± 0.02 a	0.56 ± 0.08 c	0.66 ± 0.04 b	0.76 ± 0.08 a
Total anthocyanins	mg/L malvidin	195 ± 28 f	408 ± 16 a	373 ± 11 c	240 ± 12 e	339 ± 20 d	380 ± 27 bc
Total polyphenols	mg/L gallic acid	1359 ± 62 e	1378 ± 48 de	1105 ± 45 f	1429 ± 87 cd	1979 ± 46 a	1615 ± 56 b

Data are the mean (± standard deviation) of three grape-must (each must lot was analyzed 3 folds by WineScan) samples harvested at each maturity stage (T0 = 18° Bx; T1 = 21° Bx; T3 = 23° Bx). Different letters in the same row indicate statistical significance ($p \leq 0.05$). n.d.= not detected.

It is known that during maturity anaerobic metabolism takes place in the berry (Tesniere *et al.,* 2004) and that the oxygen content of the berry flesh is almost zero (Xiao *et al.,* 2018), thus, ethanol formation can be induced inside the grape berry, albeit at very low levels. Ethanol was only detected at the third sampling time in both samples (0.09 % v/v). The presence of endogenous (physiological) ethanol can also induce the formation of acetic acid (Bianchi *et al.,* 2023; Pettinelli *et al.,* 2022), and therefore a slight increase in volatile acidity, as observed in the 08 Alto grapes. However, this slight increase could also be due to the presence of *Acetobacter* spp, *Gluconobacter* spp and, in minor quantities, lactic bacteria on the berry skin (Barata *et al.,* 2012). On the other hand, in Solicchiata grapes, volatile acidity content at the first two stages of maturity (18° and 21° Bx) was similar to the values observed in the last sampling of the 08 Alto grapes before dropping significantly. Finally, during berry maturity, glycerol increased in both vineyards as expected, due to the berry sugar increase (Bianchi *et al.,* 2022; Pettinelli *et al.,* 2022).

To summarise, at the beginning of maturity, Solicchiata had a higher acetic and lactic acid content than the 08 Alto grapes, likely due to greater oxidation of ethanol to acetaldehyde and acetic acid and, therefore, more formation of lactic acid from pyruvic acid (Santini *et al.,* 2023; Tesnière *et al.,* 1994). This hypothesis is confirmed by the fact that glycerol increased more in the Solicchiata than in the 08 Alto grapes.

In both the grape samples, malic acid was increasingly consumed with increasing maturity as expected, but we do not know if its fate is the pyruvic acid or the Krebs cycle; its decrease leads to suppose the first event (D'Onofrio *et al.,* 2019). As regards volatile acidity, in the 08 Alto grapes, the berry anaerobic metabolism was likely either less active or postponed, thus volatile acidity increased significantly only at the last sampling time. The YAN content of the 08 Alto grapes increased progressively, while in Solicchiata grapes it increased until the second sampling, then decreased, but it was always significantly higher than the that of the 08 Alto grapes. This higher YAN content in Solicchiata could be due to the higher nitrogen and organic matter content of the soil (Table 1) and this content could influence the high berry

metabolism (Bell and Henschke, 2005). Sulfate increased in the grapes from both vineyards, with similar values and in concentrations higher than those generally found in Italian grape, probably due to the high concentrations of sulfur compounds present in the soil and air. It should be noted that the Etna area is a volcanic area and its underground water is classified as both "sulfureous water", containing 45 mg/L of sulfur compounds, and "mineral", containing 7 g/L of salts (Taglieri *et al.,* 2023). Etna volcano is still active, thus the air around the volcano where the vines are grown is rich in sulfur compounds. In this macroclimate, the vine and the grape clusters absorb sulfur compounds from the soil and air.

The total anthocyanin content measured in the present study was higher than the ranges found by Giacosa *et al.* (2023) and Nicolosi *et al.*(2021): 50 – 85 mg/kg in grapes and 125 – 160 mg/L in wine respectively. At the first sampling time, total anthocyanin content was higher in the Solicchiata than in the 08 Alto grapes, but over time it was higher in 08 Alto than in Solicchiata and the values were similar at the end. In contrast to total anthocyanins, total polyphenol content was lower than that generally found in wine (Giacosa *et al.* (2023) reported 1945 – 2033 mg/L) and similar to those found in Nerello Mascalese grapes (Nicolosi *et al.,* 2021). During maturity, polyphenols decreased in the 08 Alto grapes, while in the Solicchiata ones a peak in their content was observed at the second sampling time, which was significantly higher than in 08 Alto (Table 2).

The grapes from the two vineyards differed significantly in terms of the contents of the five monoglucoside anthocyanins (Table 3). Malvidin was the most abundant, confirming previous observations (D'Onofrio *et al.,* 2021): it was 6 times higher in Solicchiata than in 08 Alto. In terms of maturity pattern, the anthocyanins (except for delphinidin) increased at a higher rate in the 08 Alto than in the Solicchiata grapes, and by the last sampling, the values were similar for all the anthocyanins. This pattern indicates that, in 08 Alto grapes, the increase in sugar content significantly affected the increase in monoglucoside anthocyanins, while in Solicchiata, in the first stage of maturity (18° Bx) the level of anthocyanins was already high and did not rise further during maturity.

TABLE 3. Monoglucoside-anthocyanins in the grape juice of grapes from 08 Alto and Solicchiata vineyards.

Anthocyanins (mg/L)	08 Alto			Solicchiata		
	T0	T1	T2	T0	T1	T2
Cyanidin 3-O-glucoside	6.24 ± 0.33 c	19.46 ± 2.70 b	30.82 ± 3.70 a	20.53 ± 1.5 b	27.85 ± 2.75 a	31.34 ± 3.20 a
Delphinidin 3-O-glucoside	13.10 ± 1.10 a	6.06 ± 0.22 e	6.94 ± 0.24 c	8.22 ± 0.24 b	6.02 ± 0.12 e	6.52 ± 0.23 d
Peonidin 3-O- glucoside	0.31 ± 0.01 f	5.94 ± 0.22 e	6.71 ± 0.27 cd	7.86 ± 0.13 a	6.47 ± 0.23 d	7.04 ± 0.10 bc
Malvidin 3-O-glucoside	26.77 ± 4.88 d	89.41 ± 4.50 c	109.25 ± 8.9 b	127.00 ± 9.50 a	134.27 ± 6.13 a	133.45 ± 6.90 a
Petunidin 3-O-glucoside	5.13 ± 0.12 e	18.03 ± 1.20 c	27.74 ± 2.71 a	15.12 ± 2.00 d	17.15 ± 2.31 cd	22.53 ± 1.87 b

Data are the mean (± standard deviation) of three grape-must samples harvested at each maturity stage (T0 = 18°Bx; T1 = 21°Bx; T3 = 23°Bx). Different letters in the same row indicate statistical significance ($p \leq 0.05$).

The positive influence of sugar on anthocyanins synthesis is well-known (Solfanelli *et al.,* 2006), particularly in grape berry (Sadras and Petrie, 2011; Tarara *et al.,* 2008). This positive coupling (sugars-anthocyanins) can be decoupled by environmental factors, such as temperature or water supply (Sadras and Moran, 2012). Regarding the pattern of total anthocyanins (Table 2), in Solicchiata there was a significant increase that was not found in monoglucoside-anthocyanins (Table 3). The ratio between total anthocyanins and total polyphenols was found to increase from 0.14 to 0.33 in the 08 Alto grapes and from 0.17 to 0.23 in Solicchiata; thus, the increase in anthocyanins at the expense of other polyphenols was higher in 08 Alto than in Solicchiata. Regarding the ratio between total monoglucoside anthocyanins and total anthocyanins, in the 08 Alto grapes it rose from 0.27 to 0.48, whereas in Solicchiata it decreased from 0.74 to 0.52.

To summarise, in 08 Alto the anthocyanins increased with maturity (especially monoglucosides), while in Solicchiata they increased slightly and the monoglucosides decreased, indicating an increase in acylated forms. However, at the last sampling time, both samples contained approximately 50 % of monoglucoside anthocyanins (0.48 vs 0.52 in 08 Alto and Solicchiata respectively).

2. GC-MS profile

As regards the GVOCs profile, Nerello Mascalese grape was characterised by a high presence of glycosylated compounds (Figure 1), with the classes glycosylated benzenoids and C_{13}- norisoprenoids in the highest concentrations. Meanwhile, the free volatiles consisted mainly in alcohols, aldehydes and acids. In the Solicchiata grapes, the concentrations of glycosylated benzenoids and C_{13}-norisoprenoids were much higher than in the 08 Alto grapes and they decreased with maturity; by contrast, in 08 Alto, the concentrations peaked at the second sampling time (Figure 1).

Of the benzenoids, the compounds in the highest concentrations were benzoic acid, 2-phenylethanol and homovanillic acid in the 08 Alto grapes (Table S1). 4-Vinylguaiacol was found at higher concentrations in Solicchiata than in 08 Alto, especially at the first sampling time. C_{13}-norisoprenoids showed the same trend as the benzenoids in both vineyard grapes: the total concentration decreased in the Solicchiata grapes, while in 08 Alto it peaked at the second sampling (Figure 1). The Solicchiata grapes showed higher concentrations of C_{13}-norisoprenoids than the 08 Alto grapes. Of the C_{13}-norisoprenoids, 3-oxo-α-ionol and vomifoliol were found in much higher concentrations than the other compounds in both grapes (Table S1). While monoterpene content was significantly lower than C_{13}- norisoprenoid content, significantly higher values were once again found in Solicchiata grapes than in 08 Alto ones (Figure 1). The monoterpene compounds in the highest content were (*E*)-8-hydroxylinalool and *cis*-pyran-linalool oxide (Table S1), with similar patterns to those of the C_{13}-norisoprenoids in both grapes. Finally, the concentrations of the alcohols (mainly C6) showed a peak at the second sampling in both the 08 Alto and Solicchiata grapes, while isoamylic alcohol content increased.

The free GVOCs contents were lower than the glycosylated ones (Figure 2). Aldehyde (especially C6 and overall (*E*)-2-hexenal) showed the highest content at T0 then decreased in 08 Alto grapes; meanwhile, alcohol content (C6) increased considerably (Table S2). In Solicchiata, the acids and alcohols showed the highest contents, which increased significantly at T1 (Figure 2)
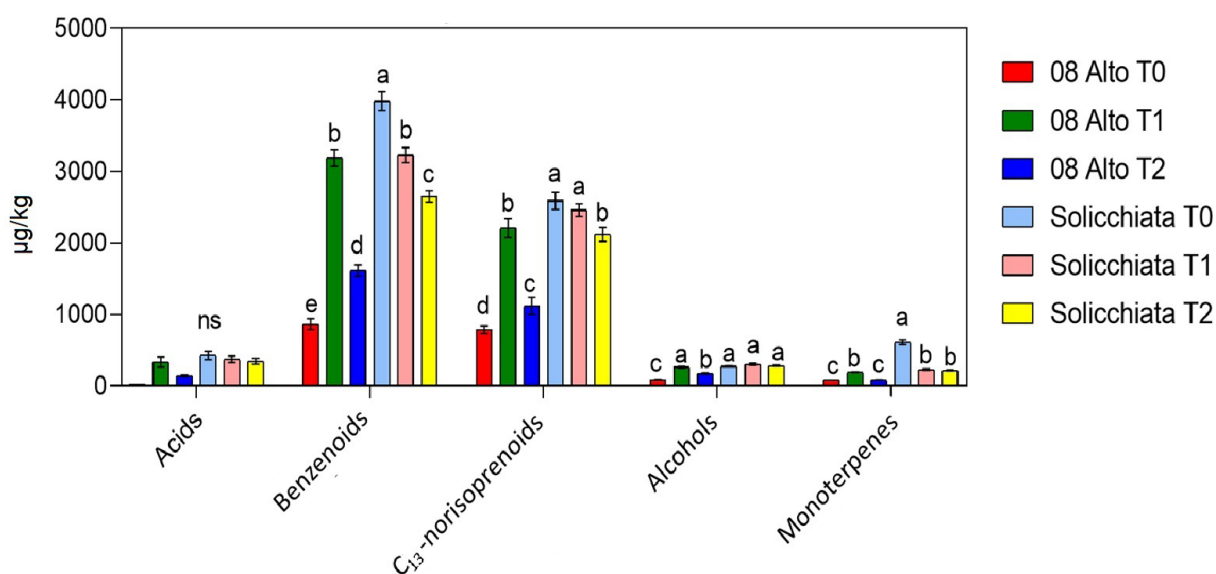

FIGURE 1. Main classes of glycosylated GVOCs in Nerello Mascalese grapes from the 08 Alto and Solicchiata vineyards at each maturity stage (T0 = 18° Bx; T1 = 21° Bx; T3 = 23° Bx). Data are the mean (bars indicate the standard deviation) of three GC analyses of three different grape samples. Different letters (within classes) indicate statistical significance (*p* ≤ 0.05).
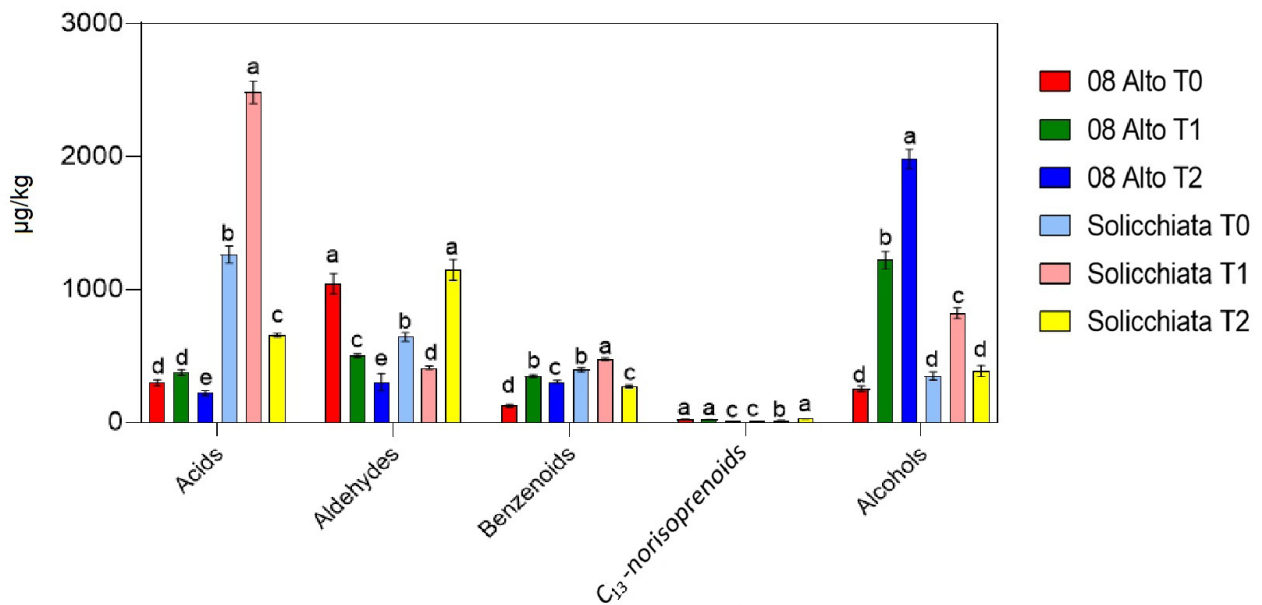
FIGURE 2. Main classes of free GVOCs of Nerello Mascalese grapes from 08 Alto and Solicchiata vineyards at each maturity stage (T0 = 18° Bx; T1 = 21° Bx; T3 = 23° Bx). Data are the mean (bars indicate the standard deviation) of three GC analyses of three different grape samples. Different letters (within classes) indicate statistical significance ($p \leq 0.05$).

In conclusion, the aromatic peak in 08 Alto occurred at 21° Bx, which concurs with the total anthocyanin peak;, meanwhile, in Solicchiata, which is generally richer in GVOCs, the highest aromatic content was at 18° Bx, which is earlier than the total anthocyanin and total polyphenol peaks.

Figure 3 shows a graphical representation of the hierarchical cluster analysis (HCA) performed using the Ward's method (PCA-based) on the main grouped GVOCs after mean centring of the original data.

The dendrogram relative to the scores (the grape samples) is coupled to a heat-map of the loadings (the grouped GVOCs), and the clustering effect is discussed as affected by the volatile dominance. The Solicchiata T0 and T1 samples form a distinct cluster, which is linked to a separate cluster of 08 Alto samples at T1 and T2. While acids (mainly fatty acids, particularly 9-oxononanoic, hexadecanoic and linoleic) and benzenoids seemed to have significantly influenced Solicchiata T1, they had a lower influence on Solicchiata T0. The 08 Alto T0 and Solicchiata T2 samples comprise a significant, **significantly tight** cluster as can be observed by the length and distance of the dendrogram branches. The scores in this cluster seem to be have been influenced by C_{13}-norisoprenoids (particularly 3-oxo-α-ionol and vomifoliol) and C6-aldehydes (hexanal and (*E*)-2-hexenal), with a slightly lower influence on 08 Alto T0 than Solicchiata T2 grapes.

The results show evidence of progressive oxidative stress on Solicchiata samples likely due to the microclimate of the Solicchiata vineyard, which is different to that of 08 Alto.

As explained in the Materials and Methods Section, Solicchiata was over 100 m higher and on a windier slope than 08 Alto, which was in a flat area close to a river. Thus, higher UV and temperature associated with wind may have stressed the Solicchiata vine and berries. The progressive increase in benzenoids (mainly benzaldehyde) from Solicchiata T0 to T1 might be associated with stress leading to aminoacidic degradation, as has already been observed by Yin *et al.* (2023). A similar increase to benzenoids it has been observed in aliphatic volatile acids (e.g., oleic, tetradecanoic, hexadecenoic, octadecanoic and octanoic acids) may be associated with an oxidation of the berry cell fat components following berry maturity (Campos-Arguedas *et al.,* 2022). In particular, polyunsaturated fatty acids (e.g., linoleic acid) are derived from phospholipidic cell membrane degradation due to lipoxygenase (LOX) activity which leads to the straight-chain aldehyde formation (Bianchi *et al.,* 2023; Liu *et al.,* 2015). Indeed, as expected, Solicchiata T2 segregation is shows C6-aldehyde dominance [hexanal and (*E*)-2-hexenal in particular], explaining the metabolic response of the activated LOX pathway.

Finally, C_{13}-norisoprenoid components (e.g., 3-oxo-α-ionol, and vomifoliol) were observed to have an impact on the Solicchiata T2 samples, which can be associated with carotenoid breakdown in response to the stressing agent (He *et al.,* 2021; Mendes-Pinto, 2009). To reinforce the stress hypothesis, even the pattern of polyphenol compounds is likely the result of stress, as polyphenol biosynthesis is induced by stressing events (Peng *et al.,* 2022). It is interesting to observe that 08 Alto samples at T0 are clustered with Solicchiata T2
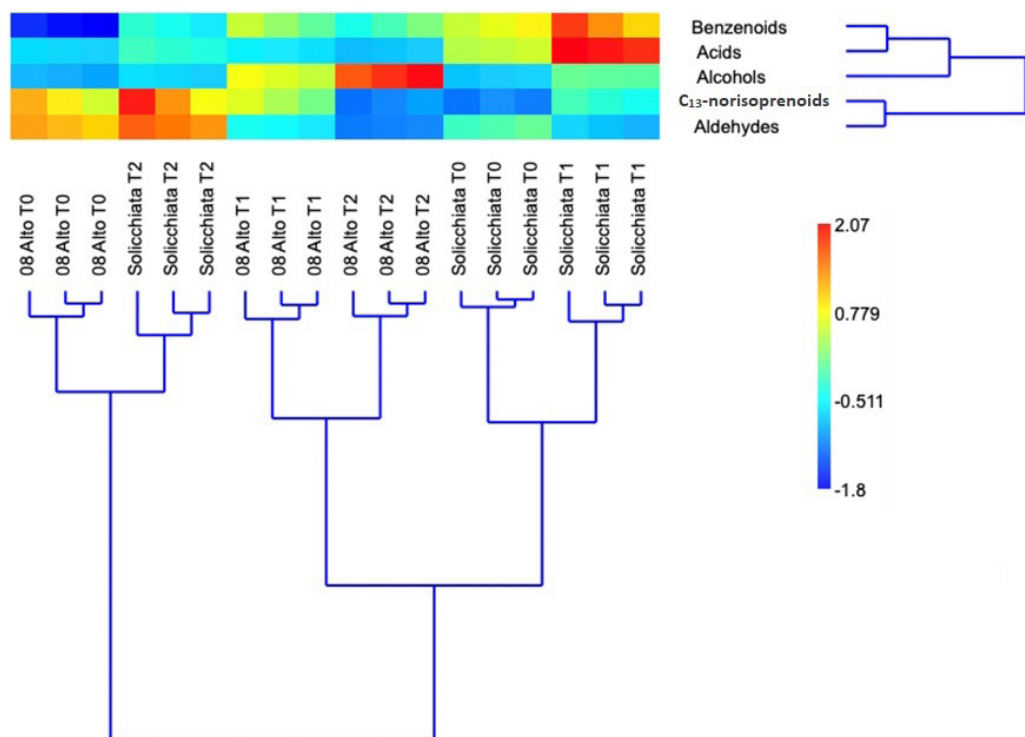
FIGURE 3. Graphical representation of the results of the HCA performed on the main categories of grouped GC-MS-detected GVOCs using the PCA-based Ward's method. The dendrogram (bottom left) represents the computed clustering of the scores, while the heat-map (top left) and the associated dendrogram (top right) show the loading influence on the score segregation together with the loading combination in this action.
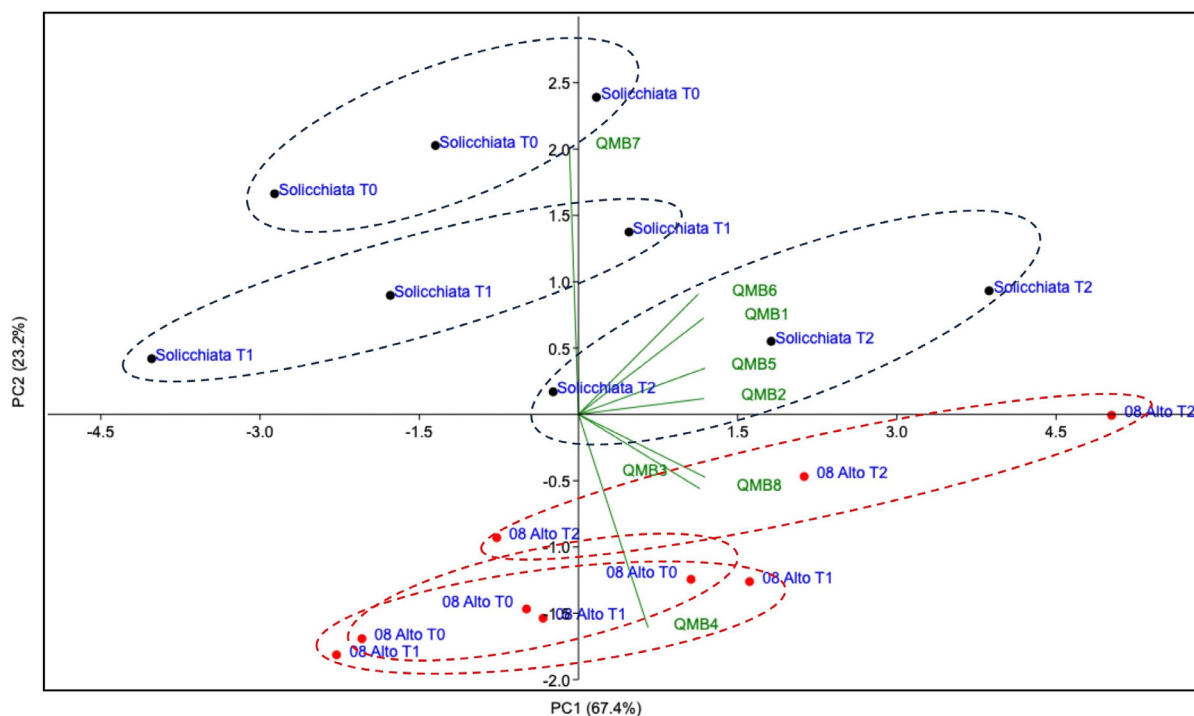

FIGURE 4. Graphical biplot of the PCA performed on E-nose detections. Scores represent the samples of the original dataset, while loadings are the variable ones. Vineyards = 08 Alto and Solicchiata and maturity stage = (T0 = 18° Bx; T1 = 21° Bx; T3 = 23° Bx).

due to the influence of aldehyde and C_{13}- norisporenoid volatiles. In 08 Alto T1 and T2, the progressive influence of the alcohols is evidenced, and the relevance of 1-hexanol can be explained as the logical consequence of the LOX pathway action, leading to the formation of C6 alcohols.

To sum up, in Solicchiata, we observed a change in GVOCs towards oxidated classes, such as C6-aldehydes and C_{13}- norisoprenoids; this partial oxidation may have been due to the higher elevation of the Solicchiata vineyard than the 08 Alto vineyard (678 m vs 555 m), thus higher UV radiation, as well as to higher canopy temperature in the case of the 08 Alto vineyard due to it being close to the river. We can thus confirm that the best aromatic maturity is at 18°

Bx in Solicchiata and at a more advanced maturity stage for 08 Alto.

3. E-nose determination

Based on the autoscaled raw data obtained from the E-nose detections, a PCA was performed and the results are represented in a graphical biplot (Figure 4).

The first two principal components describe more than the 90% of the data variability (PC1 67.4 %, PC2 23.2 %), while the rest of 3 PCs explained the residual variance under 5 % (PC3 6.1 %), thus we reported only the first 2 PCs. The Solicchiata scores can be seen to be positively correlated with PC2, being graphically located in the 1st and 4th quadrant, while 08 Alto scores are negatively correlated, occupying the
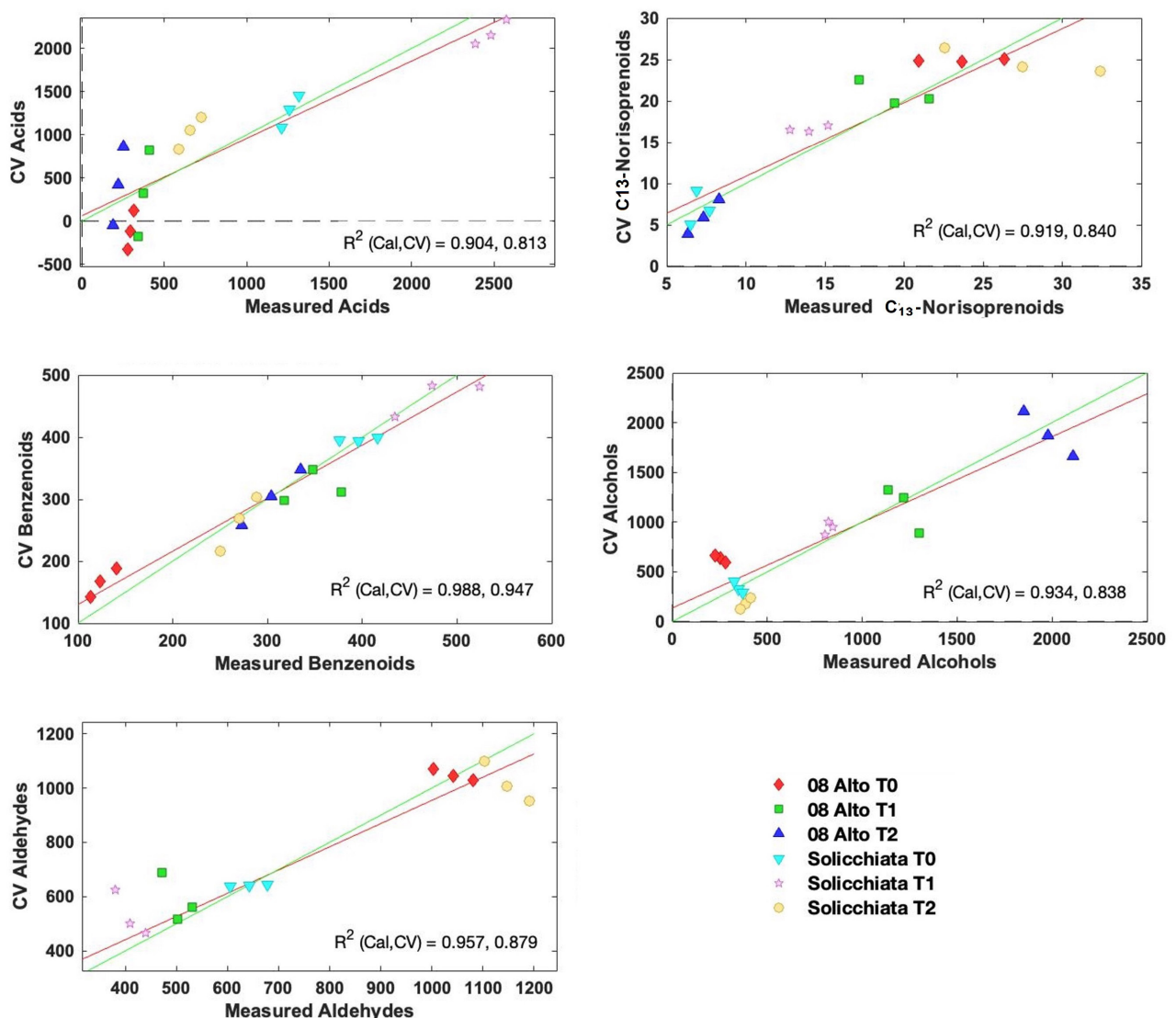

FIGURE 5. Scatterplots of the PCR performed on E-nose data (independent variable), and main categories of grouped VOCs (dependent variable). Regressions performed for acids, benzenoids, C13-norisoprenoids, aldehydes and alcohols are represented by a combination of calibrated and cross-validated (by the leave-one-out method) data. Correlation results are estimated by the determination coefficient (R^2) in correlation (R^2cal), and in cross-validation (R^2cv).

2nd and 3rd quadrant. PC1 defines quite a clear segregation of each group of samples (08 Alto and Solicchiata) according to the progressive stages of maturity. The scores move from T0 to T2 from negative to positive quadrants associated with PC1 (from 4th to 1st for Solicchiata, and from 3rd to 2nd for 08 Alto scores). On PC2, there is a marked separation of Solicchiata and 08 Alto scores for T0 and T1, and a less marked separation for T2.

The multivariate results of the E-nose measurements differ greatly to those of the GVOCs computation. This observation is to be expected given that, generally speaking, VOCs are derived from a quantitative GC-MS analysis of molecules which are surely present in the analyzed matrix; while in the case of tasting or E-nose not all the VOCs are not so certainly detected. This is because volatile molecules have different perception thresholds and are subject to chemical interactions, combinations, covering and synergy which can strongly affect their aromatic perception by the human nose, or by E-nose (Giungato *et al.*, 2018; Mastrangelo *et al.*, 2023).

It is well known that grape juice aroma is, in most cases, very similar in all the grape varieties due to the absence of fermentative processes and enzymatic or chemical reactions (Liu *et al.*, 2017) but if we run GC-MS we find a significant difference in GVOCs. An E-nose is an unspecific sensor-based device whose discriminative capacity allows it to perceive an aromatic pattern or profile in a much more similar way to a human nose than to GC-MS identification (Modesti *et al.*, 2021).

The loading effect (the action of the original dataset variables) on the PCA computations is graphically represented in the same biplot (Figure 4), making it possible to observe the influence of the different Me-porphyrin sensors on the score segregation. The 08 Alto T0 and T1 samples were significantly affected by Sensor 4 (QMB4, Rh-TPP), which is sensitive to volatile phenols, furans and lactones (Martínez-García *et al.*, 2021; Saevels *et al.*, 2004). Meanwhile, Sensors 7 (QMB7, Co-TPP) had the greatest influence on the clustering of Solicchiata T0 and T1 scores; this sensor has previously been associated with aldehydes and alcohols (Martínez-García *et al.*, 2021; Saevels *et al.*, 2004). The other sensors can be seen to be closely related to the last sampling time (T2) of 08 Alto or Solicchiata.

Finally, a PCR computation was performed in which the reduced number of original variables from the E-nose detections represented the independent variables (X-block), and the grouped GC-MS-detected volatiles the dependent ones (Y-blocks). The results are shown in Figure 5 in the form of scatterplots of the regressions. Very promising correlations can be observed for all the volatile classes, in terms of calibration (R^2cal 0.904 for acids, 0.988 for benzenoids, 0.919 for C_{13}-norisoprenoids, 0.957 for aldehydes, and 0.934 for alcohols), as well as cross-validation (R^2cv 0.813, 0.947, 0.840, 0.879, and 0.838 respectively) (Figure 5).

CONCLUSIONS

Our results demonstrate and confirm the extreme variability of vineyard response in the Etna area, despite the vineyards being close each other. In Solicchiata grapes, the concentrations of glycosylated benzenoids and C_{13}-norisoprenoids decreased with increasing maturity; by contrast, in 08 Alto, the concentration of these compounds reached a peak at the second sampling time. Progressive oxidative stress due to the specific microclimate of the Solicchiata vineyard likely influences the production of certain GVOCs with increasing maturity. Using the E-nose, the different stages of aromatic maturity were successfully clustered. The results highlight the considerable potential of the new metallo-porphyrins E-nose for use in a non-destructive approach for accurately estimating specific volatile compounds.

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