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Aromatic complexity in Verdicchio wines: a case study

Silvia Carlin[1,2], Urska Vrhovsek[1], Andrea Lonardi[3], Lorenzo Landi[3] and Fulvio Mattivi[1,4]

[1]Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach (FEM), Via E. Mach, 1 38010 S. Michele all'Adige, TN, Italy
[2]Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, Via delle Scienze 208, 33100 Udine, Italy
[3]Fazi Battaglia Winery, Via Roma 117, Castelplanio Ancona, Italy
[4]University of Trento, Department of Physics, Via Sommarive 14, 38123 Povo, TN, Italy

*Corresponding author: fulvio.mattivi@fmach.it

ABSTRACT

Aim: Verdicchio is a white wine grape variety that has been cultivated for hundreds of years in the Marche region of central Italy. Verdicchio is used to produce all kinds of dry, sweet and sparkling wines, some of which can be aged for ten or more years. This study aimed to extend knowledge of the volatile profile of Verdicchio wines and the recognition and detection of odorous molecules. We considered wines produced in multiple vintages from some of the best Cru from the Marche region, in the Castelli di Jesi Classico area.
Methods and results: Two data sets were considered: a vertical collection that included wines from different vintages, same variety, different production areas vinified by the same winery and a horizontal collection of wines from the 2016 harvest, considering different production areas, harvest times and clones produced by the same winery. Samples were analysed with GC×GC-ToF-MS, GC-MS-MS and GC-O. Comprehensive profiling with more than 1000 compounds allowed the wines produced in different areas to be separated. By GC-O analysis 48 main odorants were found. This survey led to the identification of 3-methyl-2,4-nonanedione (3-MND) that impart an anise note and an interesting content in methyl salicylate as a possible key odorant characteristic in Verdicchio.
Conclusion: The volatile profile of different Verdicchio wines from the best production areas was investigated in detail. This work confirms that it is possible to obtain wines with very different characteristics from this variety of grapes, producing premium wines with a distinctive pattern of volatiles, reproducible across several vintages and variable depending on the different location of the vineyard and winemaking techniques. Young wines are characterised by fruity, thiolic notes, while wines aged for longer are distinguished by their norisoprenoids content and by anise and balsamic notes, which can be attributed to the presence of 3-MND and to methyl salicylate released by precursors. With a derivatisation GC-MS-MS method it was possible to quantify 3-MND in the range of 10–50 ng L^{-1}. With the use of GC-O, 48 potentially odour-active compounds were found for this wine. Analysis of the compounds after hydrolysis confirmed that a high amount of methyl salicylate characterised this variety. Methyl salicylate has a balsamic note from wintergreen oil that is often perceived in aged Verdicchio tasting.

KEYWORDS

Verdicchio, solid phase extraction, solid-phase-microextraction, aroma

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INTRODUCTION

The aroma of a wine is influenced by the action of several different compounds on the sensory organs. These compounds are produced through metabolic pathways during the ripening and harvesting of grapes and during the fermentation and storage of wine (Rapp and Mandery, 1986; Rapp and Versini, 1995). Of these factors, it is well known that the grape varieties gives characteristic aromas. This varietal aroma allows the classification and even the labelling of wines, so it is important to know the typical aromatic pattern of a wine variety in order to ensure its quality (Marais, 1983).

Verdicchio is a white grape variety grown almost exclusively in the Marche region in central Italy. Genetic analysis has shown a very close relationship between Verdicchio, "Trebbiano di Soave" and "Trebbiano di Lugana or Turbiana" (Costacurta *et al.*, 2003). The genetic origin of Verdicchio and therefore of Trebbiano di Soave, is however still being debated. One hypothesis, based on the documented 15th century migration of farmers from the province of Verona in northern Italy to the Marche, partially depopulated by the plague, is now the most accredited. The settlers are thought to have brought their vines with them and thus it is surmised that they originally came from the Veneto region. In a recent work (Ghidoni *et al.*, 2010) it was reported that the three varieties are genetically identical, at least as far as the analysed part of the genome is concerned, but they could be traced to three different biotypes of the same variety, in relation to phenotypical traits related to the environment. Indeed, the length of the separation would appear to justify some olfactory differences between Trebbiano di Soave and Verdicchio, over and beyond the demonstrated genetic identity (Vantini *et al.*, 2003). Verdicchio is not characterised by a large quantity of terpenes. Indeed, in analysis carried out in the past, a very low content of the main monoterpenes was observed. The floral terpene note could derive from the Ho-terpendiol I (about 20 µg L^{-1}), also present in bound forms, which could then be released over time (Nicolini *et al.*, 2003). What was instead observed was the presence of a characterising compound, methyl salicylate, which in this variety can reach 45 µg L^{-1} in free forms and more than 500 µg L^{-1} in bound forms, as reported for Trebbiano di Soave and Verdicchio (Versini *et al.*, 2005). When added to wine, it is described as providing a floral note, slightly balsamic, tending to cinnamon-chestnut honey (Bauer *et al.*, 2008). A study involving Trebbiano di Lugana wines (genetically similar to Trebbiano di Soave and Verdicchio) highlighted that the grapes also contain the precursors of sulphured aromatic compounds, in particular 3-mercaptohexanol and 3-mercapto hexyl acetate, which could impart tropical and citrus fruit scents to the wines (Mattivi *et al.*, 2012). During the tasting of this wine, notes of anise/fennel are sometimes perceived and even balsamic scents in aged wines. One of the objectives of this work was to understand which compounds could contribute to these descriptors. For this reason we used gas chromatography–olfactometry (GC-O), a powerful tool for the study of chemical compounds responsible for wine aroma (van Ruth, 2001).

MATERIALS AND METHODS

1. Wine samples

1.1. Sample set 1 (vertical collection)

For the preliminary survey, four types of wines were obtained using Verdicchio grapes grown in different areas but in the same Italian region (Marche) and produced by Fazi Battaglia winery. A sample of 24 Verdicchio wines from different vintages – 11 Massaccio (2002–2015), eight San Sisto (2001–2015), three Titulus (2013–2015) and three Le Moie (2008–2015) – were analysed with SPME and GC×GC-ToF-MS. Massaccio is obtained from the highest and most south-facing areas, San Sisto derives from colder areas with clayish soil, Le Moie is obtained in vineyards closer to the sea on sandy soils and Titulus is produced with grapes grown in the 300 hectares of estate-vineyards, subdivided into 12 distinct vineyards with different exposure.

1.2. Sample set 2 (horizontal collection, 2016 harvest)

Wines from each of the three different areas (Massaccio, San Sisto and Le Moie) were analysed. In addition, in the case of San Sisto, wines produced from three different harvesting data were produced: early harvest (H1) on 23 September, normal harvest (H2) after 1 week and late harvest on 5 October (H3). Furthermore, for Massaccio, five experimental wines were produced with three different clones (VCR-3, VL-50, R-2) and from separate harvest of vines of high and low vigour. All the wines were

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obtained following a strictly controlled protocol. The grapes were harvested manually and subjected to cold maceration at 10 °C for 3 h in reductive conditions, then pressed at 0.2 bar. The obtained juices were inoculated with selected yeasts *Saccharomyces cerevisiae* (Zymaflore X5 Laffort). Fermentation was performed under a controlled temperature gradient, starting at 12 °C and gradually increasing up to 19°C at the end of fermentation. After fermentation, two "batonnages" per week were performed. The wines stayed 7 months on the lees and no malolactic fermentation was done.

Two additional wines were produced with modified winemaking techniques: one with skin contact and the other, known as "Arkezia", was designed produce a sweet wine using grapes infected with noble rot.

The volume of the experimental wines was 6 hl for the three clones and 2.3 hl for Arkezia. All other thesis were produced at real scale, i.e. ≥ 40 hl.

Three different bottles of each of the 2016 wines were extracted with the solid phase extraction (SPE) technique and both free and bound (after enzymatic hydrolysis) forms were analysed. The most volatile compounds were analysed with the SPME technique, 3-methyl-2,4-nonanedione (3-MND) was quantified with SPME after derivatisation and the SPE extract was used for GC-O analysis.

2. Solid phase extraction (SPE ENV+) procedure

50 mL of wine were extracted with SPE using ENV+ cartridges, 1 g (Biotage, Sweden). The cartridge was pre-conditioned with 15 mL of methanol followed by 20 mL of water. The aqueous extract was loaded onto the cartridge, which was then washed with 15 mL of water. The free aromatic compounds were eluted from the cartridge with 30 mL of dichloromethane and the bound aromatic compounds (i.e. glycosides) with 30 mL of methanol. The free volatiles were collected in a 100-mL flask and 60 mL of pentane was added, followed by the addition of anhydrous Na_2SO_4 to remove water. Subsequently, prior to analysis the whole fraction was carefully concentrated up to 200 µL using a Vigreux column. Methanol was eliminated under vacuum and the residue solubilised in 5 mL of a citrate buffer pH 5, while the glycosidically bound fraction was hydrolysed with 400 µL of a commercial glycosidase rich enzyme (70 mg mL^{-1} Rapidase AR 2000 DSM) at 40 °C for 12 h. The mixture with the aglycons was eluted through an ENV+ 1 g cartridge previously activated with 5 mL of methanol and 10 mL of milliQ water and the free compounds were collected with 10 mL of dichloromethane. The relative amount of each volatile was expressed as µg/L of n-heptanol.

3. Solid phase microextraction (SPME) for 3-methyl-2,4-nonanedione (3-MND) determination after PFBHA (O-(2,3,4,5,6-Pentafluorobenzyl)hydroxylamine) derivatisation

5 mL of wine were put in a 20 mL headspace vial with 20 µL of IS (4-methyl-3-penten-2one d_{11} 10 µg L^{-1}) and 400 µL of a o-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (PFBHA) water solution (20 g L^{-1}) was added to the vial. The method was adapted from Moreira *et al.* (2013) and Saison *et al.* (2009) The PFBHA solution was prepared daily in ultrapure water at 20 g L^{-1}. The IS was prepared in ethanol at 1 g L^{-1} and further diluted at 10 µg L^{-1} The vials were equilibrated for 20 min at room temperature, after that a 65 µm PDMS/DVB was inserted into the headspace for 40 min at 50 °C. Standard solutions were prepared in ethanol at 1 g L^{-1} and stored at 4 °C. For the calibration curves, seven different concentrations of 3-MND, from 10 ng L^{-1} to 400 ng L^{-1}, were spiked in a white wine.

4. Solid phase microextraction (SPME) and GC-MS-MS method for 3-MND quantification

GC analysis was performed on a Trace GC Ultra gas chromatograph coupled with an XLS Tandem mass spectrometer. The system was equipped with a Pal autosampler (CTC Analytics AG, Zwingen, Switzerland). GC separation was performed on a 30 m VF Wax column with an internal diameter of 0.25 mm and a film thickness of 0.25 µm (Agilent). Supelco (Bellafonte, USA) PDMS/DVB 65 µm fibre was exposed in the sample for 40 min at 50 °C and then desorbed into the GC liner, set at 250 °C in splitless/surge mode for 3 min. The temperature programme was 80 °C held for 4 min after injection, 8 °C/min up to 220 °C, held for 1 min and 15 °C/min up to 270°C, held for 5 min. Helium was used as the carrier gas at a constant flow rate of 1.2 mL min^{-1}. The mass spectro-

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meter was operated in electron impact (EI) ionisation mode at 70 eV. The filament current was 50 µA. The temperature of the transfer line was 250°C and argon (99.9998% purity) was used as the collision gas, with collision cell pressure of 1.5 mTorr. Data acquisition and analysis were performed using the Xcalibur Workstation software supplied by the manufacturer. In order to determine the retention time and the characteristic mass fragments of 3-MND, full scan analysis (m/z 40–300) was performed. The retention times were 29.62 and 29.91 min for monosubstituted 3-MND and RT 35.83 and 36.39 and 36.81 for disubstituted 3-MND. For quantitative analysis, we selected the transitions m/z 168>71 (10 eV) for monosubstituded 3-MND and m/z 363>181 (10 eV) for disubstituted 3-MND. In addition, the transitions m/z 267>86 (10 eV) and 307>181 (10 eV) were also used as qualifiers for mono and disubstituted 3-MND, respectively. For the internal standard the transition m/z 122>74 and m/z 122> 78 (10 eV) were used. For quantification, the chosen transitions were monitored in multiple reaction monitoring (MRM) mode and the peak area ratios of 3-MND to 4-methyl-3-penten-2one d_{11} (internal standard) were calculated on the basis of the compound concentration.

Quantification was performed using the internal standard (IS) method. For the calibration curves, seven different concentrations of 3-MND from 10 ng L^{-1} to 400 ng L^{-1} were spiked in a white wine. The repeatability of the method was checked by running 10 samples spiked with two different concentrations (20 ng L^{-1} and 100 ng L^{-1}) and the cv% was <13%.

5. Solid phase microextraction (SPME) and two-dimensional (GC×GC) gas chromatography with time-of-flight mass spectrometry (TOF-MS)

1.5 g of sodium chloride was added to a 20 mL headspace vial, followed by 1 mL of wine spiked with 50 µL of 2-octanol as internal standards (1 mg L^{-1}). Quality control (QC) samples consisting of equal proportions of each sample were taken at the beginning of the run (n=5) and then after every tenth sample. GC×GC-TOF-MS analysis of wines was performed using a gas chromatograph (GC) Agilent 6890N (Agilent Technologies), coupled with a LECO Pegasus IV time-of-flight mass spectrometer (TOF-MS) (Leco Corporation, St. Joseph, MI, USA) equipped with a Gerstel MPS autosampler (Gerstel GmbH & Co. KG). Samples were incubated for 5 min at 35 °C and volatiles were extracted with a DVB/CAR/PDMS coating of 50/30 µm and 2-cm long SPME fibre (Supelco Sigma-Aldrich, Milan, Italy) for 20 min, desorbed for 3 min at 250 °C in splitless mode. The fibre was reconditioned between each sample for 7 min at 270 °C. Helium was used as a carrier gas at a flow of 1.2 mL min^{-1}. The GC oven was equipped with a VF Wax ms 30 m × 0.25 mm column with 0.25 µm film thickness (Agilent Technologies) for the first dimension (1D) and a Rxi 17 Sil MS 1.5 m × 0.15 mm column with 0.15 µm film thickness (Restek, Bellefonte, PA, USA) for the second dimension (2D). Oven temperature was held at 40 °C for 3 min and ramped at the rate of 6 °C/min to 250 °C, where it was held for 5 min before returning to the initial conditions. The secondary oven temperature was held at 5 °C above the temperature of the primary oven throughout the chromatographic run. The modulator was offset by +15 °C in relation to the secondary oven and modulation time was 7 s. The ion source temperature was set at 230 °C and electron ionisation at 70 eV. Spectra were collected in a mass range of m/z 40–350 with an acquisition rate of 200 spectra/s and acquisition delay of 120 s.

6. Gaschromatograpy-olfactometry and mass spectrometry (GC-O-MS)

1 µL of SPE extract was analysed with a Trace GC Ultra chromatograph, coupled with a ISQ QD single quad mass spectrometer (Thermo Scientific, Milan, Italy), equipped with a splitless injector maintained at 250 °C; the split vent was opened 0.5 min post injection. Compounds were analysed with a VF Wax (Agilent J&W, Folsom, CA, USA) column (30 m × 0.25 mm i.d., 0.25 µm film thickness) in scan mode. The carrier gas was helium. The oven temperature was programmed to rise from 50 °C to 250 °C at 2.5 °C/min and then be kept at 250 °C for 10 min. To assess the olfactory potential of the extracts, the column was connected to a GC-O port Olfactory Port (GL Sciences Tokyo, Japan) maintained at 220 °C. The effluent was diluted with a large volume of air (20 mL min^{-1}) prehumidified with an aqueous solution. For aroma extract dilution analysis (AEDA), the concentrated fraction was diluted stepwise (1:3) with pentane:dichloromethane (2:1) to obtain dilutions 1:9, 1:27, 1:81, 1:243. Complete aroma

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extract dilution analysis (AEDA) was performed by five trained panellists. Sniffing of dilutions was continued until no odorant could be detected by GC-MS-O. Each odorant was thus assigned a flavour dilution factor (FD factor) representing the last dilution in which the odorant was still detectable. The analysis was repeated in duplicate by each assessor.

7. Volatile compounds identification

Volatile compounds identification was achieved by comparing retention times and mass spectra with those of pure standards when available and with mass spectra from NIST05 and Wiley libraries. Linear retention indices (relative to n-alkanes C10 to C28) were calculated and compared to those from literature.

8. Statistical data elaboration

Data from 1D-GC-MS and GC×GC-TOF-MS analyses were processed using multivariate techniques. PLS-DA and heatmaps were generated using MetaboAnalyst v. 3.0 (http://www.metaboanalyst.ca), created at the University of Alberta, Canada (Xia *et al.*, 2015).

9. Sensory test

In order to understand the contribution of 3-MND to the Verdicchio aroma, the standard was added to a "neutral" Verdicchio (3-MND not detectable). The concentrations tested were 10 ng L^{-1}, 25 ng L^{-1}, 50 ng L^{-1}, 100 ng L^{-1} and 500 ng L^{-1}. A blank wine, without additions, was also included in the tasting.

RESULTS AND DISCUSSION

The initial exploration was carried out using four different types of Verdicchio wines (Massaccio, San Sisto, Le Moie and Titulus) by analysing a series of vintages and using SPME with GC×GC-ToF-MS. Two-dimensional analysis made it possible to find many compounds characterising the different types of Verdicchio. Indeed, it is possible to notice clear separation in the PLS-DA (partial least squares discriminant analysis) plot (Figure 1). As shown in the graph, the first component can separate types of Titulus, especially old vintages, from other wines and San Sisto, Massaccio and Le Moie are separated by the second component.

The volatile compounds determining this separation were whisky lactone, 5-methyl-furfural, 2-furancarboxaldehyde, 2,4,5-trimethyl-1,3-dioxolane, benzofuran and 2-methyl-thiophene for the San Sisto samples. The first three compounds derive from the use of wood, while dioxolane is a compound correlated with ageing. Le Moie wines were characterised by a higher content of norisorenoids such as β-damascenone and safranal. Titulus wines were richer in other norisoprenoids, such as TDN and also in some sesquiterpenes such as alpha calacorene and monoterpenes such as alpha-terpineol and alpha terpinolene. Wines from the Massaccio areas were the richest in benzenacetaldehyde and linalool. San Sisto and Massaccio were also the richest in 4-methyl 1-pentanol and 2-acetyl furanone. It is also possible to observe that the typical trend of TDN increases over the years in Massaccio wines (Figure 2). This compound can derive from different precursors linked to sugar molecules and is formed by hydrolysis during ageing (Versini *et al.*, 2001; Winterhalter and Schreier, 1994; Winterhalter, 1991); pH, temperature and presence of oxygen are important parameters in the formation and degradation of TND (Silva Ferreira *et al.*, 2004).

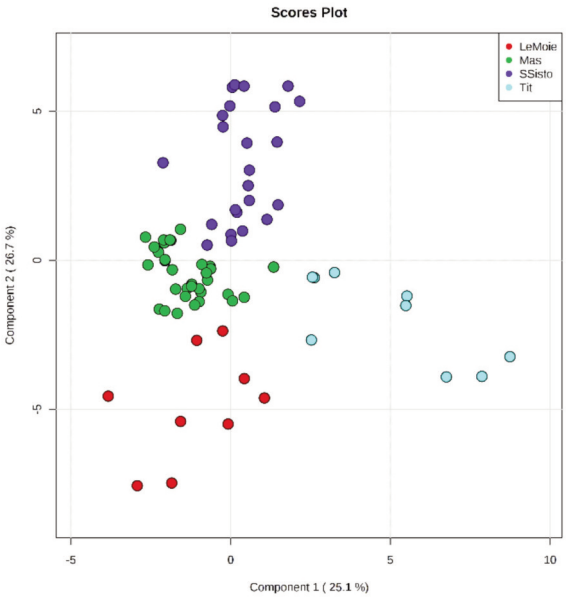


FIGURE 1. Partial least squares discriminant analysis (PLS-DA) scatterplot of Verdicchio wines from grapes grown in different areas. Eleven vintages of Massaccio (2002–2015), eight vintages of San Sisto (2001–2015), three vintages of Titulus (2013–2015) and three vintages of Le Moie (2008–2015).
The score scatter each point represents the vintages (each vintage with three technical replicates).

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After analysing the vertical collection of old vintages as a survey study, a horizontal collection of Verdicchio wines produced in 2016 were analysed in depth. In this case, several different tests were carried out in order to understand the volatile profile of this variety. Results from SPE and SPME analyses are shown in Tables 1 and 2. As the data refer to a single vintage, no definitive conclusions can be drawn, although the wines from Massaccio, San Sisto and Le Moie areas were again distinguishable (Figure 3, heatmap free). Le Moie wine was distinguished by a higher content of hexyl acetate, octanoic and decanoic ethyl ester and the corresponding acids, by the highest amount of Ho-terpendiol I and of 3-methyl-2,4-nonanedione. Le Moie was also the area richest in the bound form. Massaccio was higher in norisoprenoids such as TDN and its precursor riesling acetale, methyl salicylate, homofuraneol and 2-(H)-pyran-2,6(3H)-dione. Some of these compounds will be also discussed in the GC-O section. Samples from San Sisto H1 were the richest in benzyl alcohol, methionol, 2-methyltetrahydrothiophen-3-one and 4-vinyl-guaiacol. It was also clear that the amount of terpenes increased from San Sisto H1 to San Sisto H3. The latter was indeed the richest in terpenoids such as linalool, citronellol, limonene and alpha-terpineol, both in the free and in the bound forms (Figure 4) and in β-damascenone. TDN, vitispirane and riesling acetale reached their maximum in the H2 harvest data and then decreased slightly in H3; the same trend was reported in previous studies (Coelho *et al.*, 2007).

The most vigorous vines resulted in a wine with a higher content of C6 compounds such as 1-hexanol, *trans* and *cis* 3-hexenol, *trans* 2-hexenol and hexyl acetate, while C_{13} norisoprenoids, especially TDN, were higher in the wine from less vigorous vines. This is in accordance with the literature (Kwasniewski *et al.*, 2010), where an increase in solar radiation leads to an increase of its precursor carotenoids.

The wines from different clones differed in the content of thiols (data not reported in this paper) and also in the content of methyl salicylate. Late harvested Arkezia, obtained from grapes with noble rot and a withering stage, was characterised by the presence of marker compounds for *Botrytis* infection, such as benzaldehyde, acetic acid, furfural and terpinen-4-ol. (Fedrizzi *et al.*, 2011)

However, it will be necessary to confirm these preliminary observations made for the 2016 vintage by considering other vintages.

To better understand the sensorial impact of some compounds in Verdicchio aroma, SPE extracts from the three different areas were injected into GC-O and sniffed by six expert panellists: 48 main odorants were found in the SPE extract (Table 3). What is perceived in the glass is a mixture of odour and taste molecules, which may combine to act in a suppressive or additive manner, or synergistically (Frijters and Schifferstein, 1994). In GC-O, by contrast, the odorants are delivered to the olfactory epithelia as single entities, which simplifies the recognition task. Aroma extract dilution analysis (AEDA) was used to determine the relative odour potency of compounds present in the sample extract.

Aroma compounds with a higher FD value (>3) likely contributed considerably to overall Verdicchio wine aroma. In particular, we



FIGURE 2. Increase of TDN (µg/L) content during ageing in 11 vintages of Verdicchio wines produced in the Massaccio area, 2002–2015.

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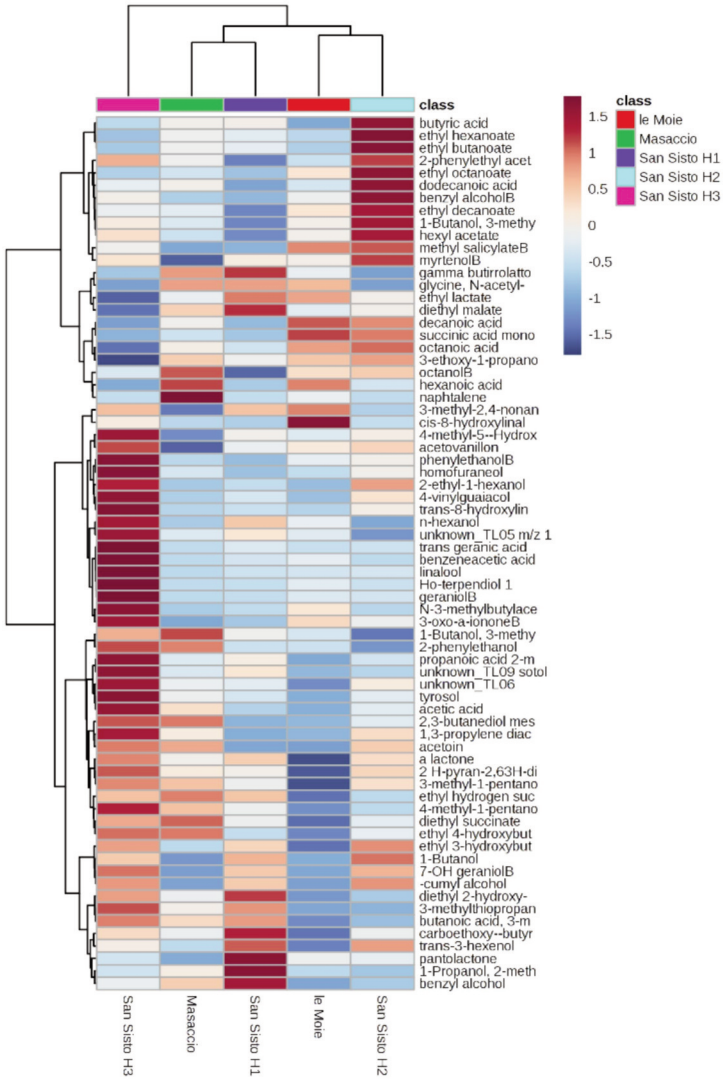


FIGURE 3. Hierarchical clustering analysis performed using volatile aroma compound profiles of 2016 wines produced from grapes from three areas (Le Moie, Massaccio, San Sisto) and three harvest data (San Sisto) (Ward algorithm and Euclidean distance analysis using MetaboAnalyst v. 3.0).
The heatmap was generated using 70 compounds. The rows in the heatmap represent compounds and the columns indicate samples. The colours of the heatmap cells indicate the abundance of compounds across different samples. The colour gradient, ranging from dark blue through white to dark red, represents low, middle, and high abundance of a compound.

observed that the most important compounds in terms of sensorial impact were ethyl 2-methylpropanoate, with notes of apple fruit, ethyl hexanoate with a bilberry-fruity note, β-damascenone with a baked apple note, furaneol and homofuraneol with a cotton candy scent, vanillin with a characteristic sweet pastry note, phenylacetic acid associated with honey-withered flower, one unknown compound with a toasted almond smell, isovaleric acid with a cheese odour, β-ionone with a violet note and methional and methionol with a boiled potato odour. In some wines, a passion fruit/tropical note was also found at the retention index very close to 3-mercapto-hexanol. Moreover, in all samples a liquorice note was present, probably associated with the compound pantolactone, together with a clove/spice note given by 4-vinylguaiacol and a clear celery/vegetal scent, probably due to sotolone.

Furthermore, we focused our attention on the anise note noticeable in some vintages of Verdicchio. GC-O analysis made it possible to find the area where the six expert panellists sensed this note. In the literature, the compounds that can contribute to this note are *trans* and *cis* anethole, estragol (Zeller and Rychlik, 2007), ethyl hexanoate (San-Juan *et al.*, 2010),

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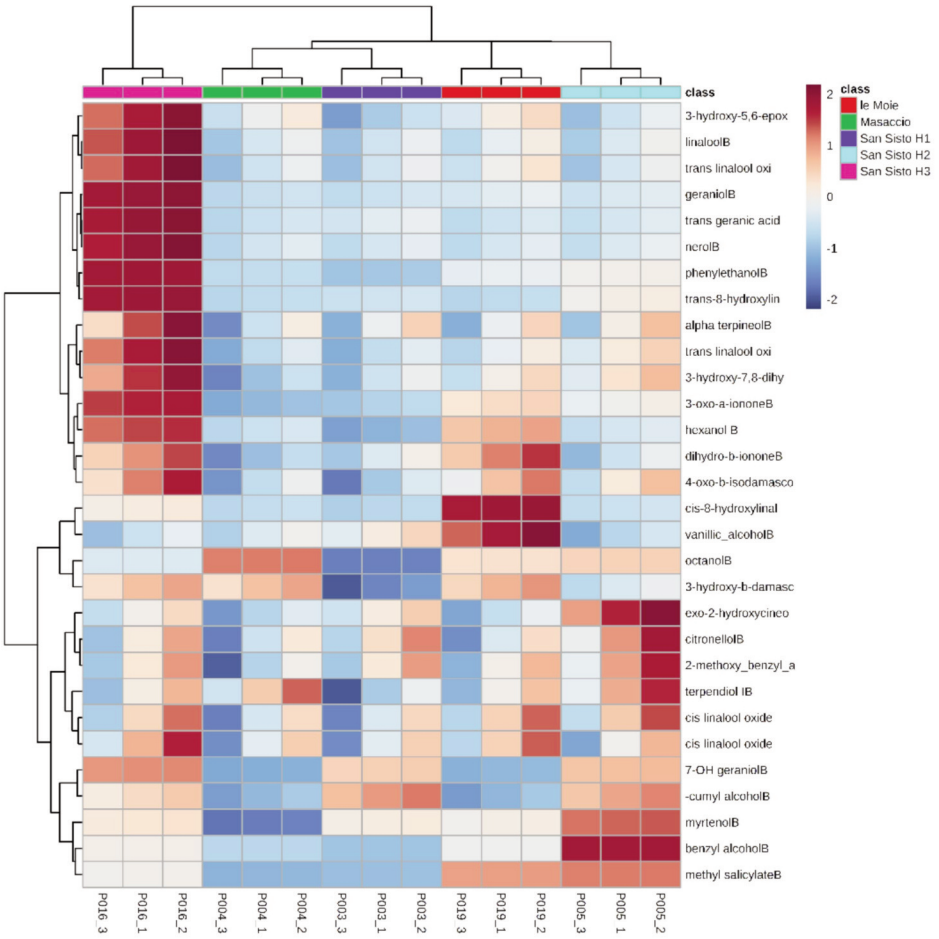


FIGURE 4. Hierarchical clustering analysis performed using volatile aroma compound profiles of 2016 wines produced from grapes from three areas (Le Moie, Massaccio, San Sisto) and three harvest data (San Sisto) (Ward algorithm and Euclidean distance analysis using MetaboAnalyst v. 3.0).
The heatmap was generated using 30 most significant bound compounds after enzymatic hydrolysis (the highest Fisher ratios). The rows in the heatmap represent compounds and the columns indicate samples. The colours of the heatmap cells indicate the abundance of compounds across different samples. The colour gradient, ranging from dark blue through white to dark red, represents low, middle and high abundance of a compound.

R carvone (Pinar *et al.*, 2017) and 3-methyl-2,4 nonanedione (Pons *et al.*, 2008). Except for ethyl hexanoate, which is eluted early, all the other compounds showed an RI on a polar column between 1687 and 1800. Analysis of the pure standards led to identification of the 3-methyl-2,4 nonanedione (3-MND) compound responsible for the anise note. Other researchers have reported an anise-like note in the same area that has been tentatively identified (Martí *et al.*, 2003; Peťka *et al.*, 2006). This compound was found in wine by French researchers (Pons *et al.*, 2008); it was related to the plum note in prematurely aged red wines and was also correlated with a loss of fruitiness (Pons *et al.*, 2013). 3-MND is an intriguing compound that has recently emerged as the most potent known agonist for the human receptor OR1A1, with a submicromolar half-maximal effective concentration (Geithe *et al.*, 2017). These results further emphasise the controversial role of this compound, whose presence at variable concentrations has previously been described as reminiscent of mint, anise, fruit kernels and prunes (Pons *et al.*, 2008) and has been associated with prematurely aged wines, oxidised soybean oil (Kao *et al.*, 1998) and freshly brewed green tea (Guth and Grosch, 1993). The odour description of this compound in wine model solution is strong, depending on its concentration, as reported by Pons *et al.* (2008). When the concentration is 100 ng L^{-1} it

TABLE 1. Concentration (means ± standard deviations) and odour thresholds (OPT) in µg/L except * in mg/L as equivalent of n-heptanol of free volatile compounds in 2016 Verdicchio wines.

Free aroma compounds	OPT°	RI exp	RI lit	Harvest date / Areas					Vigour		Clones			Vinification	
				le Moie	Massaccio	San Sisto H1	San Sisto H2	San Sisto H3	Low vigour	High vigour	VCR3	VL50	R2	Late harvest	Skin maceration
ethyl butanoate*	0,02	1030	1030	0.32 ± 0.02	0.37 ± 0.02	0.35 ± 0.02	0.5 ± 0.02	0.31 ± 0.01	0.3 ± 0.01	0.3 ± 0.01	0.33 ± 0.02	0.35 ± 0.02	0.29 ± 0.01	0.19 ± 0.01	0.35 ± 0.02
1-propanol, 2-methyl*	40	1087	1060	2.95 ± 0.1	3.56 ± 0.12	5.03 ± 0.18	2.79 ± 0.1	3.12 ± 0.11	3.02 ± 0.11	2.38 ± 0.08	3.86 ± 0.14	3.48 ± 0.12	5.19 ± 0.18	8.1 ± 0.28	4.97 ± 0.17
1-butanol, 3-methyl-, acetate*	0,03	1122	1120	1.81 ± 0.07	1.64 ± 0.06	1.1 ± 0.04	2.63 ± 0.1	1.88 ± 0.07	2.79 ± 0.11	3.54 ± 0.13	2.31 ± 0.09	2.64 ± 0.1	2.55 ± 0.1	0.82 ± 0.03	1.41 ± 0.05
1-butanol*	150	1135	1130	0.25 ± 0.01	0.21 ± 0.01	0.64 ± 0.02	0.72 ± 0.03	0.59 ± 0.02	0.59 ± 0.02	0.49 ± 0.02	0.21 ± 0.01	0.19 ± 0.01	0.2 ± 0.01	0.16 ± 0.01	0.65 ± 0.02
limonene	10	1197	1199	16.9 ± 0.59	3.75 ± 0.13	2.74 ± 0.1	2.3 ± 0.08	2.09 ± 0.07	0.97 ± 0.03	1.58 ± 0.06	31.79 ± 1.11	22.26 ± 0.78	3.65 ± 0.13	4.51 ± 0.16	1.7 ± 0.06
1-butanol, 3-methyl-*	30	1205	1221	77.4 ± 3.48	92.9 ± 4.18	80.6 ± 3.63	66.6 ± 2.99	88.1 ± 3.97	94.7 ± 4.26	86.6 ± 3.9	95.5 ± 4.3	103.9 ± 4.68	110.1 ± 4.95	97.1 ± 4.37	92.6 ± 4.17
ethyl hexanoate*	0,014	1234	1236	1.14 ± 0.05	1.31 ± 0.05	1.25 ± 0.05	1.84 ± 0.08	1.08 ± 0.04	0.9 ± 0.04	0.96 ± 0.04	1.09 ± 0.04	1.23 ± 0.05	1.08 ± 0.04	0.55 ± 0.02	1.01 ± 0.04
hexyl acetate*	1,5	1270	1272	0.14 ± 0.004	0.12 ± 0.004	0.08 ± 0.003	0.21 ± 0.01	0.16 ± 0.01	0.18 ± 0.01	0.25 ± 0.01	0.14 ± 0.005	0.18 ± 0.01	0.21 ± 0.01	0.01 ± 0.001	0.08 ± 0.003
acetoin	150000	1279	1268	9.81 ± 0.38	27.21 ± 1.06	10.41 ± 0.41	24.73 ± 0.96	29.35 ± 1.14	70.82 ± 2.76	88.28 ± 3.44	39.15 ± 1.53	19.97 ± 0.78	9.23 ± 0.36	16.28 ± 0.63	7.65 ± 0.3
4-methyl-1-pentanol		1312	1328	20.9 ± 0.75	37.21 ± 1.34	31.34 ± 1.13	26.65 ± 0.96	44.37 ± 1.6	43.98 ± 1.58	42.79 ± 1.54	35.48 ± 1.28	49.38 ± 1.78	37.4 ± 1.35	24.57 ± 0.88	29.65 ± 1.07
3-methyl-1-pentanol	830	1324	1341	53.1 ± 1.91	97.37 ± 3.51	85.02 ± 3.06	92.07 ± 3.31	104.69 ± 3.77	94.98 ± 3.42	87.87 ± 3.16	99.05 ± 3.57	111.71 ± 4.02	85.21 ± 3.07	61.59 ± 2.22	79.1 ± 2.85
ethyl lactate*	154	1340	1326	3.98 ± 0.16	3.03 ± 0.12	4.18 ± 0.17	3.21 ± 0.13	1.6 ± 0.07	1.02 ± 0.04	0.95 ± 0.04	2.32 ± 0.1	2.87 ± 0.12	3.04 ± 0.12	1.98 ± 0.08	3.36 ± 0.14
n-hexanol*	8	1354	1356	1.36 ± 0.05	1.28 ± 0.05	1.45 ± 0.06	1.24 ± 0.05	1.58 ± 0.06	1.05 ± 0.04	1.16 ± 0.05	1.51 ± 0.06	1.57 ± 0.06	1.86 ± 0.07	0.45 ± 0.02	1.75 ± 0.07
trans-3-hexenol	400	1363	1361	50.7 ± 1.87	67.3 ± 2.49	103.2 ± 3.82	97.1 ± 3.59	80.8 ± 2.99	63.1 ± 2.34	65.5 ± 2.42	71.1 ± 2.63	67.1 ± 2.48	67.3 ± 2.49	14.3 ± 0.53	73.0 ± 2.7
3-ethoxy-1-propanol*	0,1	1371	1389	0.57 ± 0.02	0.56 ± 0.02	0.48 ± 0.01	0.61 ± 0.02	0.25 ± 0.01	0.17 ± 0.01	0.16 ± 0	0.55 ± 0.02	0.45 ± 0.01	0.58 ± 0.02	0.09 ± 0	0.74 ± 0.02
cis-3-hexenol		1386	1379	45.5 ± 1.64	31.93 ± 1.15	35.62 ± 1.28	33.69 ± 1.21	23.39 ± 0.84	39.95 ± 1.44	53.19 ± 1.91	58.71 ± 2.11	53.03 ± 1.91	86.18 ± 3.1	12.41 ± 0.45	116.1 ± 4.18
trans-2-hexen-1-ol		1407	1410	8.98 ± 0.31	10.55 ± 0.37	22.0 ± 0.77	10.37 ± 0.36	9.29 ± 0.33	6.56 ± 0.23	10.05 ± 0.35	11.12 ± 0.39	12.58 ± 0.44	12.58 ± 0.44	12.5 ± 0.44	10.27 ± 0.36
ethyl octanoate*	0,02	1437	1435	1.87 ± 0.08	1.67 ± 0.08	1.55 ± 0.07	2.29 ± 0.1	1.59 ± 0.07	1.32 ± 0.06	1.32 ± 0.06	1.41 ± 0.06	1.54 ± 0.07	1.47 ± 0.07	0.63 ± 0.03	1.18 ± 0.05
acetic acid*	200	1453	1430	0.25 ± 0.01	0.4 ± 0.02	0.29 ± 0.01	0.33 ± 0.01	0.55 ± 0.02	0.21 ± 0.01	0.13 ± 0.01	0.67 ± 0.03	0.61 ± 0.02	0.81 ± 0.03	1.14 ± 0.04	0.39 ± 0.02
2-ethyl-1-hexanol		1491	1485	38.2 ± 1.14	39.59 ± 1.19	41.95 ± 1.26	57.28 ± 1.72	63.66 ± 1.91	58.8 ± 1.76	51.53 ± 1.55	43.47 ± 1.3	31.08 ± 0.93	33.26 ± 1	36.65 ± 1.1	67.01 ± 2.01
benzaldehyde	1508	1506	1508	15.9 ± 0.51	9.7 ± 0.31	29.18 ± 0.93	25.47 ± 0.82	19.06 ± 0.61	9.6 ± 0.31	9.63 ± 0.31	14.73 ± 0.47	11.13 ± 0.36	11.31 ± 0.36	40.56 ± 1.3	13.28 ± 0.42
ethyl 3-hydroxybutanoate*		1511	1499	0.22 ± 0.01	0.31 ± 0.01	0.42 ± 0.02	0.47 ± 0.02	0.46 ± 0.02	0.43 ± 0.02	0.41 ± 0.02	0.28 ± 0.01	0.31 ± 0.01	0.3 ± 0.01	0.18 ± 0.01	0.53 ± 0.02
2-methyltetrahydrothiophen-3-one		1514	1538	16.1 ± 0.52	18.06 ± 0.58	27.74 ± 0.89	27.26 ± 0.87	23.75 ± 0.76	35.48 ± 1.14	38.48 ± 1.23	13.96 ± 0.45	18.33 ± 0.59	17.67 ± 0.57	11.9 ± 0.38	11.73 ± 0.38
2,3-butanediol meso*	100	1530	1525	1.39 ± 0.04	2.46 ± 0.07	1.37 ± 0.04	1.76 ± 0.05	2.54 ± 0.08	1.51 ± 0.05	2.01 ± 0.06	2.57 ± 0.08	2.49 ± 0.07	2.81 ± 0.08	3.05 ± 0.09	1.41 ± 0.04
propanoic acid		1540	1546	56.1 ± 2.58	63.79 ± 2.93	70.63 ± 3.25	71.68 ± 3.3	75.6 ± 3.48	82.79 ± 3.81	64.79 ± 2.98	95.41 ± 4.39	73.97 ± 3.4	78.33 ± 3.6	88.64 ± 4.08	69.07 ± 3.18
linalool	25	1551	1542	11.8 ± 0.59	7.37 ± 0.37	10.7 ± 0.53	12.09 ± 0.6	56.37 ± 2.81	14.37 ± 0.72	16.19 ± 0.81	7.29 ± 0.36	7.65 ± 0.38	8.17 ± 0.41	5.1 ± 0.25	33.14 ± 1.65
1-octanol		1554	1552	17.5 ± 0.53	20.03 ± 0.6	25.93 ± 0.78	13 ± 0.39	13.58 ± 0.41	10.62 ± 0.32	9.54 ± 0.29	17.2 ± 0.52	11.35 ± 0.34	13.61 ± 0.41	15.14 ± 0.45	23.56 ± 0.71
propanoic acid 2-methyl- *		1567	1583	0.36 ± 0.01	0.46 ± 0.02	0.51 ± 0.02	0.44 ± 0.02	0.71 ± 0.03	0.44 ± 0.02	0.37 ± 0.01	0.94 ± 0.03	0.52 ± 0.02	0.62 ± 0.02	1.53 ± 0.06	0.49 ± 0.02
cis-4-hydroxymethyl-2-methyl-1,3-dioxolane		1602	1623	2.14 ± 0.07	4.75 ± 0.15	1.91 ± 0.06	3.14 ± 0.1	8.31 ± 0.27	10.17 ± 0.33	8.5 ± 0.27	12.15 ± 0.39	3.62 ± 0.12	3.3 ± 0.11	24.85 ± 0.8	2.59 ± 0.08
γ–butirrolattone*	35	1611	1650	0.33 ± 0.01	0.4 ± 0.02	0.43 ± 0.02	0.27 ± 0.01	0.29 ± 0.01	0.16 ± 0.01	0.14 ± 0.01	0.44 ± 0.02	0.48 ± 0.02	0.32 ± 0.01	0.56 ± 0.02	0.39 ± 0.02
butyric acid*	0,17	1623	1600	0.97 ± 0.04	1.11 ± 0.04	1.11 ± 0.04	1.34 ± 0.05	1.03 ± 0.04	0.76 ± 0.03	0.83 ± 0.03	1.13 ± 0.04	1.01 ± 0.04	0.9 ± 0.04	0.66 ± 0.03	1.03 ± 0.04
phenylacetaldehyde		1626	1612	4.72 ± 0.15	3.58 ± 0.11	3.66 ± 0.12	3.16 ± 0.1	4.27 ± 0.14	3.43 ± 0.11	3.96 ± 0.13	4.65 ± 0.15	4.55 ± 0.15	4.8 ± 0.15	44.69 ± 1.43	4 ± 0.13
ethyl decanoate*		1641	1637	1.39 ± 0.05	1.23 ± 0.04	0.87 ± 0.03	1.81 ± 0.06	1.26 ± 0.04	1.45 ± 0.05	1.17 ± 0.04	1.28 ± 0.04	1.33 ± 0.04	1.16 ± 0.04	0.64 ± 0.02	0.96 ± 0.03
butanoic acid, 3-methyl- *	0,03	1667	1642	0.54 ± 0.03	0.69 ± 0.04	0.74 ± 0.04	0.58 ± 0.03	0.75 ± 0.04	0.73 ± 0.04	0.69 ± 0.04	0.85 ± 0.05	0.64 ± 0.04	0.78 ± 0.04	1.08 ± 0.06	0.95 ± 0.05
diethyl succinate*	6	1672	1667	1.96 ± 0.06	4.37 ± 0.14	3.29 ± 0.11	3.16 ± 0.1	4.06 ± 0.13	3.47 ± 0.11	3.46 ± 0.11	3.2 ± 0.1	2.87 ± 0.09	2.37 ± 0.08	3.21 ± 0.1	2.5 ± 0.08
α-terpineol		1686	1684	10.3 ± 0.36	7.92 ± 0.28	6.36 ± 0.22	5.07 ± 0.18	19.9 ± 0.7	6.62 ± 0.23	6.72 ± 0.24	9.13 ± 0.32	8.47 ± 0.3	10.44 ± 0.37	4.47 ± 0.16	18.62 ± 0.65
3-methylthiopropanol*	1	1705	1725	0.45 ± 0.01	0.68 ± 0.02	0.86 ± 0.03	0.47 ± 0.01	0.92 ± 0.03	1.16 ± 0.03	1.11 ± 0.03	0.51 ± 0.02	0.56 ± 0.02	0.66 ± 0.02	1.31 ± 0.04	1.04 ± 0.03
naphtalene		1715	1728	9 ± 0.23	29.52 ± 0.74	4.65 ± 0.12	4.63 ± 0.12	4.94 ± 0.12	3.94 ± 0.1	4.65 ± 0.12	29.36 ± 0.73	24.88 ± 0.62	28.48 ± 0.71	24.69 ± 0.62	5.25 ± 0.13
1,3-propylene diacetate (t.i.)*		1730	-	0.61 ± 0.02	0.74 ± 0.02	0.6 ± 0.02	0.77 ± 0.02	0.92 ± 0.03	0.98 ± 0.03	1.11 ± 0.03	0.68 ± 0.02	0.52 ± 0.02	0.77 ± 0.02	0.76 ± 0.02	0.89 ± 0.03
α-cumyl alcohol		1750	1779	24.2 ± 0.7	24.36 ± 0.71	66.48 ± 1.93	76.54 ± 2.22	75.76 ± 2.2	71.3 ± 2.07	74.63 ± 2.16	26.82 ± 0.78	25.47 ± 0.74	24.24 ± 0.7	25.48 ± 0.74	71.83 ± 2.08
methyl salicylate	50	1754	1759	30.5 ± 1.13	44.79 ± 1.66	41.73 ± 1.54	35.28 ± 1.31	33.51 ± 1.24	26.51 ± 0.98	25.34 ± 0.94	37.91 ± 1.4	24.9 ± 0.92	21.12 ± 0.78	19.16 ± 0.71	23.21 ± 0.86
methyl-4-hydroxybutanoate		1758	-	3.14 ± 0.08	5.44 ± 0.14	3.33 ± 0.08	4.44 ± 0.11	6.04 ± 0.15	4.33 ± 0.11	3.85 ± 0.1	11 ± 0.28	6.36 ± 0.16	6.35 ± 0.16	42.69 ± 1.07	15.37 ± 0.38
ethyl 4-hydroxybutanoate*		1799	1827	1.16 ± 0.03	2.88 ± 0.08	1.75 ± 0.05	2 ± 0.06	2.9 ± 0.08	1.66 ± 0.05	1.3 ± 0.04	7.07 ± 0.21	4.03 ± 0.12	3.25 ± 0.09	10.53 ± 0.31	3.77 ± 0.11
2-phenylethyl acetate*	0,25	1802	1803	0.2 ± 0.01	0.25 ± 0.01	0.12 ± 0.01	0.37 ± 0.01	0.32 ± 0.01	0.71 ± 0.02	0.83 ± 0.02	0.39 ± 0.01	0.43 ± 0.01	0.43 ± 0.01	0.18 ± 0.01	0.14 ± 0
4-(methylthio)-1-butanol		1833	1812	8.21 ± 0.26	7.93 ± 0.25	11.87 ± 0.38	12.16 ± 0.39	11.98 ± 0.38	8.97 ± 0.29	8.62 ± 0.28	6.81 ± 0.22	6.41 ± 0.21	6.69 ± 0.21	4.47 ± 0.14	12.51 ± 0.4
hexanoic acid*	0,42	1840	1830	4.41 ± 0.2	4.64 ± 0.21	2.82 ± 0.13	3.16 ± 0.15	2.55 ± 0.12	1.95 ± 0.09	2.48 ± 0.11	3.02 ± 0.14	3.59 ± 0.17	3.98 ± 0.18	1.87 ± 0.09	2.27 ± 0.1
N-(3-methylbutyl)acetamide*		1855	1855	0.12 ± 0.004	0.02 ± 0.001	0.04 ± 0.001	0.03 ± 0.001	0.29 ± 0.01	0.01 ± 0.001	0.01 ± 0.001	0.04 ± 0.002	0.03 ± 0.001	0.04 ± 0.001	2.11 ± 0.07	0.35 ± 0.01
benzyl alcohol*		1863	1876	0.16 ± 0.01	0.31 ± 0.02	0.4 ± 0.02	0.19 ± 0.01	0.25 ± 0.01	0.28 ± 0.02	0.28 ± 0.02	0.23 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.04 ± 0	0.48 ± 0.03
2-phenylethanol*	50	1895	1893	24.1 ± 0.92	33.26 ± 1.26	24.15 ± 0.92	19.31 ± 0.73	34.58 ± 1.31	35.05 ± 1.33	38.9 ± 1.48	37.03 ± 1.41	39.96 ± 1.52	43.35 ± 1.65	52.9 ± 2.01	28.07 ± 1.07
Ho-terpendiol 1		1943	1957	27.61 ± 0.97	8.61 ± 0.3	10.86 ± 0.38	13.4 ± 0.47	152.14 ± 5.32	11.22 ± 0.39	11.03 ± 0.39	10.82 ± 0.38	9.57 ± 0.33	7.03 ± 0.25	18.84 ± 0.66	49.57 ± 1.73
trans-2-hexenoic_acid		1960	1971	29.39 ± 0.65	38.16 ± 0.84	33.5 ± 0.74	28.51 ± 0.63	42.92 ± 0.94	45.43 ± 1	46.45 ± 1.02	80.17 ± 1.76	53.91 ± 1.19	78.05 ± 1.72	10 ± 0.22	105.09 ± 2.31
2-(H)-pyran-2,6(3H)-dione (t.i.) *		1979	-	0.56 ± 0.02	0.92 ± 0.03	0.89 ± 0.03	0.97 ± 0.03	1.12 ± 0.04	1 ± 0.04	0.93 ± 0.03	1 ± 0.04	0.81 ± 0.03	0.85 ± 0.03	0.36 ± 0.01	0.77 ± 0.03
pantolactone		2013	2033	56.6 ± 1.87	45.61 ± 1.51	78.56 ± 2.59	55.41 ± 1.83	53.02 ± 1.75	40.41 ± 1.33	45.76 ± 1.51	77.44 ± 2.56	55.93 ± 1.85	79.05 ± 2.61	88.98 ± 2.94	85.13 ± 2.81
diethyl malate*		2029	2070	2.02 ± 0.07	2.51 ± 0.09	3.14 ± 0.11	2.19 ± 0.07	1.13 ± 0.04	1.08 ± 0.04	1.12 ± 0.04	1.37 ± 0.05	1.8 ± 0.06	1.69 ± 0.06	1.19 ± 0.04	1.82 ± 0.06
octanoic acid*	0,5	2053	2043	7.78 ± 0.3	6.84 ± 0.27	6.29 ± 0.25	8.07 ± 0.31	5.01 ± 0.2	4.57 ± 0.18	5.25 ± 0.2	4.9 ± 0.19	6.33 ± 0.25	6.07 ± 0.24	2.12 ± 0.08	4.5 ± 0.18
homofuraneol	40	2075	2090	11.7 ± 0.42	14.6 ± 0.53	7.23 ± 0.26	20.24 ± 0.73	48.22 ± 1.74	76.67 ± 2.76	28.96 ± 1.04	92.52 ± 3.33	40.59 ± 1.46	21.94 ± 0.79	33.86 ± 1.22	8.69 ± 0.31
unknown m/z 114 *		2094	-	0.26 ± 0.01	0.26 ± 0.01	0.27 ± 0.01	0.23 ± 0.01	0.32 ± 0.01	0.3 ± 0.01	0.29 ± 0.01	0.44 ± 0.01	0.33 ± 0.01	0.44 ± 0.01	0.23 ± 0.01	0.28 ± 0.01
diethyl 2-hydroxy-3-methylsuccinate		2137	-	49.6 ± 1.49	83.9 ± 2.52	128 ± 3.84	63.8 ± 1.91	114.4 ± 3.43	83.1 ± 2.49	129.7 ± 3.89	97.3 ± 2.92	69 ± 2.07	89.2 ± 2.68	92.7 ± 2.78	250.3 ± 7.51
4-vinylguaiacol		2175	2175	66.4 ± 2.12	78.5 ± 2.51	108 ± 3.46	160.6 ± 5.14	278.4 ± 8.91	162.8 ± 5.21	148.9 ± 4.76	95.2 ± 3.05	140.5 ± 4.5	95.9 ± 3.07	142.5 ± 4.56	113.7 ± 3.64
carboethoxy-γ-butyrolactone*		2211	2241	0.67 ± 0.04	0.99 ± 0.06	1.36 ± 0.08	1 ± 0.06	1.11 ± 0.07	0.88 ± 0.05	1.02 ± 0.06	0.58 ± 0.04	0.74 ± 0.05	0.77 ± 0.05	0.51 ± 0.03	1.1 ± 0.07
decanoic acid*	1	2269	2257	3.88 ± 0.12	3 ± 0.09	2.36 ± 0.07	3.72 ± 0.11	2.18 ± 0.07	2.71 ± 0.08	3.03 ± 0.09	2.45 ± 0.07	2.95 ± 0.09	2.74 ± 0.08	1.21 ± 0.04	2.11 ± 0.06
succinic acid monoethylester		2267	2256	50.5 ± 1.52	35.38 ± 1.06	32.39 ± 0.97	48.41 ± 1.45	30.81 ± 0.92	40 ± 1.2	39.82 ± 1.19	31.51 ± 0.95	38.94 ± 1.17	33.58 ± 1.01	15.68 ± 0.47	29.9 ± 0.9
4-methyl-5-(β-hydroxyethyl)thiazole *		2285	2300	0.2 ± 0.001	0.16 ± 0.001	0.21 ± 0.001	0.22 ± 0.01	0.28 ± 0.01	0.15 ± 0	0.22 ± 0.01	0.54 ± 0.01	0.13 ± 0	0.38 ± 0.01	0.09 ± 0.001	0.06 ± 0.001
ethyl hydrogen succinate*		2373	2367	18.9 ± 0.57	41.36 ± 1.24	37.52 ± 1.13	27.02 ± 0.81	37.68 ± 1.13	34.26 ± 1.03	33.73 ± 1.01	41.64 ± 1.25	41.62 ± 1.25	31.7 ± 0.95	40.16 ± 1.2	26.66 ± 0.8
dodecanoic acid*		2472	2465	0.31 ± 0.01	0.35 ± 0.01	0.25 ± 0.01	0.5 ± 0.02	0.33 ± 0.01	0.5 ± 0.02	0.5 ± 0.02	0.27 ± 0.01	0.24 ± 0.01	0.27 ± 0.01	0.15 ± 0.01	0.23 ± 0.01
benzeneacetic acid	2500	2539	2534	41.5 ± 0.95	30.62 ± 0.7	36.48 ± 0.84	30.58 ± 0.7	100.1 ± 2.3	30.84 ± 0.71	32.97 ± 0.76	36.72 ± 0.84	31.34 ± 0.72	57.85 ± 1.33	233.01 ± 5.36	112.17 ± 2.58
acetovanillon*		2607	2626	0.11 ± 0.001	0.09 ± 0.001	0.11 ± 0.001	0.12 ± 0.001	0.13 ± 0.001	0.14 ± 0.001	0.14 ± 0.001	0.12 ± 0.001	0.1 ± 0.001	0.1 ± 0.001	0.19 ± 0.001	0.22 ± 0.01
tyrosol*		2990	2985	2.92 ± 0.07	4.89 ± 0.12	4.26 ± 0.11	4.56 ± 0.11	8.8 ± 0.22	9.98 ± 0.25	9.65 ± 0.24	6.33 ± 0.16	5.07 ± 0.13	6.66 ± 0.17	6.96 ± 0.17	4.62 ± 0.12
C13-norisoprenoids SPME															
β-damascenone	0,05	1801	1820	4.86 ± 0.002	3.08 ± 0.003	1.57 ± 0.001	2.8 ± 0.003	3.3 ± 0.001	5.24 ± 0.002	5.28 ± 0.003	3.37 ± 0.001	3.18 ± 0.002	3.57 ± 0.001	3.32 ± 0.003	2.62 ± 0.001
VTP (sum of isomers)	800	1524	1514	2.45 ± 0.1	2.93 ± 0.11	3.93 ± 0.15	2.87 ± 0.11	0.5 ± 0.02	2.82 ± 0.11	2.5 ± 0.1	2.26 ± 0.09	2.37 ± 0.09	1.82 ± 0.07	3.74 ± 0.15	3.6 ± 0.14
riesling acetale		1656	1638	2.5 ± 0.11	2.68 ± 0.11	2.51 ± 0.11	2.51 ± 0.11	2.56 ± 0.11	2.96 ± 0.12	2.87 ± 0.12	2.66 ± 0.11	2.6 ± 0.11	2.74 ± 0.12	2.3 ± 0.1	2.46 ± 0.1
TDN	2	1739	1743	1.06 ± 0.04	3.3 ± 0.13	1.45 ± 0.06	2.15 ± 0.09	1.52 ± 0.06	3.98 ± 0.16	3.1 ± 0.12	3.37 ± 0.13	1.82 ± 0.07	2.63 ± 0.11	1.33 ± 0.05	3.14 ± 0.13
SPME with derivatization															
3-methyl-2,4-nonanedione (ng/L)	0,016	1732	1723	77 ± 10.01	16 ± 2.08	67 ± 8.71	35 ± 4.55	68 ± 8.84	24 ± 3.12	28 ± 3.64	30 ± 3.9	29 ± 3.77	38 ± 4.94	60 ± 7.8	54 ± 7.02

Three different areas (Le Moie, Massaccio, San Sisto), three harvest times (San Sisto H1, H2, H3), low and high vigour, three different clones and two different vinifications (late harvest and whitering process, skin maceration) RI exp and lit on polar column.

is perceived as minty, while at around 1 µg L^{-1} it recalls anise, kernel and prune and at 10 µg L^{-1} the perception is of anise.

In order to understand the contribution of this compound to the Verdicchio aroma, 3-MND was added to a "neutral" Verdicchio (3-MND not detectable). The tasting was carried out by ten trained people (eight males, two females), who determined that the fruity note was masked only by the addition of 500 ng L^{-1} of 3-MND, as the anise note predominated in this wine. The samples preferred by the panellists were those with a 3-MND concentration of between 25 and 50 ng L^{-1}. No oxidative note was detected in any sample. In Verdicchio wines 3-MND instead seems capable of imparting a positive and characteristic note.

Unfortunately, a well-perceived smell on the nose (olfactometry) is frequently not detected by chemical detectors (FID or MS). This is the case

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TABLE 2. Concentration (means ± standard deviations) in µg/L except * in mg/L as equivalent of n-heptanol of bound compounds in 2016 Verdicchio wines.

Bound aroma compounds	RI exp	RI lit	le Moie	Harvest date				Vigour		Clones			Vinification	
				Areas										
				Massaccio	San Sisto H1	San Sisto H2	San Sisto H3	Low vigour	High vigour	VCR3	VL50	R2	Late harvest	Skin maceration
n-hexanol	*1354*	*1356*	54.7 ± 9.4	44 ± 7.5	39.3 ± 6.7	44.7 ± 7.6	59.5 ± 10.2	34.2 ± 5.8	30.9 ± 5.3	33.2 ± 5.7	38.5 ± 6.6	28.3 ± 4.8	285.5 ± 48.8	73.2 ± 12.5
n-octanol*	*1554*	*1552*	0.47 ± 0.03	0.56 ± 0.03	0.27 ± 0.02	0.49 ± 0.03	0.4 ± 0.02	0.41 ± 0.02	0.43 ± 0.03	0.49 ± 0.03	0.53 ± 0.03	0.65 ± 0.04	0.45 ± 0.03	0.24 ± 0.01
trans-linalool oxide furan	*1444*	*1429*	3.13 ± 0.38	1.8 ± 0.22	1.85 ± 0.22	2.02 ± 0.24	8.4 ± 1.01	2.34 ± 0.28	2.38 ± 0.29	2.46 ± 0.3	2.39 ± 0.29	2.3 ± 0.28	3.83 ± 0.46	6.85 ± 0.82
cis-linalool oxide furan	*1463*	*1454*	3.97 ± 0.46	3.04 ± 0.35	3.02 ± 0.35	3.33 ± 0.39	4.33 ± 0.5	3.85 ± 0.45	3.95 ± 0.46	3.94 ± 0.46	4.35 ± 0.5	3.93 ± 0.46	4.28 ± 0.5	5.74 ± 0.67
linalool	*1546*	*1545*	1.11 ± 0.16	0.46 ± 0.06	0.31 ± 0.04	0.67 ± 0.09	7.62 ± 1.07	0.76 ± 0.11	0.84 ± 0.12	0.6 ± 0.08	0.47 ± 0.07	0.45 ± 0.06	0.74 ± 0.1	4.26 ± 0.6
tricyclo octane (t.i.)	*1679*	-	0.54 ± 0.04	0.32 ± 0.02	0.25 ± 0.02	0.41 ± 0.03	0.61 ± 0.04	0.6 ± 0.04	0.51 ± 0.04	0.7 ± 0.05	0.57 ± 0.04	0.5 ± 0.04	1.53 ± 0.11	1.36 ± 0.1
α-terpineol	*1686*	*1698*	6.04 ± 0.79	5.48 ± 0.71	6.07 ± 0.79	6.38 ± 0.83	8.38 ± 1.09	7.41 ± 0.96	8.23 ± 1.07	8.7 ± 1.13	7.34 ± 0.95	8.82 ± 1.15	7.48 ± 0.97	21.85 ± 2.84
trans-linalool oxide pyran	*1727*	*1716*	7.31 ± 1.02	6.01 ± 0.84	6.11 ± 0.86	8.41 ± 1.18	12.72 ± 1.78	8.18 ± 1.15	9.22 ± 1.29	6.83 ± 0.96	7.9 ± 1.11	7.31 ± 1.02	10.57 ± 1.48	11.84 ± 1.66
cis-linalool oxide pyran	*1755*	*1781*	5.04 ± 0.66	3.93 ± 0.51	3.98 ± 0.52	5.15 ± 0.67	4.97 ± 0.65	2.87 ± 0.37	3.6 ± 0.47	3.62 ± 0.47	4.12 ± 0.54	4.16 ± 0.54	3.69 ± 0.48	3.43 ± 0.45
citronellol	*1762*	*1772*	1.89 ± 0.17	1.68 ± 0.15	2.87 ± 0.26	3.75 ± 0.34	2.6 ± 0.23	2.3 ± 0.21	4.99 ± 0.45	1.28 ± 0.12	0.89 ± 0.08	2.71 ± 0.24	1.89 ± 0.17	2.82 ± 0.25
nerol	*1790*	*1819*	4.5 ± 0.5	4.37 ± 0.48	4.58 ± 0.5	4.91 ± 0.54	17.82 ± 1.96	5.96 ± 0.66	5.56 ± 0.61	5.38 ± 0.59	4.47 ± 0.49	4.74 ± 0.52	4.42 ± 0.49	10.63 ± 1.17
geraniol	*1847*	*1831*	12.97 ± 1.56	9.75 ± 1.17	10.31 ± 1.24	12.05 ± 1.45	37.13 ± 4.46	11.88 ± 1.43	12.39 ± 1.49	10.57 ± 1.27	10.46 ± 1.26	10.75 ± 1.29	8.8 ± 1.06	25.07 ± 3.01
exo-2-hydroxycineole	*1849*	*1861*	19.66 ± 2.56	19.3 ± 2.51	21.39 ± 2.78	24.8 ± 3.22	21.07 ± 2.74	23.03 ± 2.99	25.52 ± 3.32	22.2 ± 2.89	22.85 ± 2.97	24.43 ± 3.18	37.87 ± 4.92	50.07 ± 6.51
HO-terpendiol I	*1943*	*1957*	7.66 ± 0.46	8.51 ± 0.51	6.47 ± 0.39	8.92 ± 0.54	7.81 ± 0.47	8.14 ± 0.49	8.52 ± 0.51	8.26 ± 0.5	8.86 ± 0.53	10.06 ± 0.6	7.02 ± 0.42	5.02 ± 0.3
trans-8-hydroxylinalool	*2264*	*2277*	35.95 ± 3.24	36.16 ± 3.25	39.18 ± 3.53	49.07 ± 4.42	81.57 ± 7.34	57.1 ± 5.14	65.36 ± 5.88	41.81 ± 3.76	50.07 ± 4.51	46.42 ± 4.18	62.85 ± 5.66	82.47 ± 7.42
7-OH geraniol (t.i.)	*2299*	-	18.86 ± 1.32	16.15 ± 1.13	63.77 ± 4.46	68.83 ± 4.82	78.97 ± 5.53	71.89 ± 5.03	61.96 ± 4.34	15.75 ± 1.1	16.83 ± 1.18	17.45 ± 1.22	17.18 ± 1.2	81.43 ± 5.7
cis-8-hydroxylinalool	*2302*	*2315*	51.37 ± 4.11	14.53 ± 1.16	12.99 ± 1.04	15.75 ± 1.26	26.09 ± 2.09	12.88 ± 1.03	14.68 ± 1.17	16.5 ± 1.32	18.93 ± 1.51	15.41 ± 1.23	28.54 ± 2.28	30.12 ± 2.41
trans-geranic acid	*2301*	*2289*	6.77 ± 0.81	6.35 ± 0.76	8.42 ± 1.01	7.32 ± 0.88	26.25 ± 3.15	10.48 ± 1.26	8.69 ± 1.04	7.38 ± 0.89	8.72 ± 1.05	8.4 ± 1.01	5.97 ± 0.72	31.12 ± 3.73
myrtenol (t.i.)	*2500*	-	171.4 ± 11.31	136.6 ± 9.02	173.1 ± 11.42	197 ± 13	176.1 ± 11.62	161.1 ± 10.63	170.3 ± 11.24	142.7 ± 9.42	159.2 ± 10.51	187.5 ± 12.38	272.5 ± 17.99	353.1 ± 23.3
3-hydroxy-β-damascone	*2512*	*2558*	32.26 ± 3.87	31.77 ± 3.81	21.97 ± 2.64	27.24 ± 3.27	31.75 ± 3.81	33.84 ± 4.06	35.37 ± 4.24	23.08 ± 2.77	24.44 ± 2.93	28.36 ± 3.4	26.25 ± 3.15	38.76 ± 4.65
3-oxo-α-ionone	*2603*	*2582*	67 ± 7.37	52.56 ± 5.78	55.31 ± 6.08	62.52 ± 6.88	79.17 ± 8.71	102.37 ± 11.26	90.43 ± 9.95	85.87 ± 9.45	73.09 ± 8.04	91.79 ± 10.1	116.1 ± 12.77	150.4 ± 16.54
dihydro-β-ionone (t.i.)	*2642*	-	17.69 ± 1.77	11.89 ± 1.19	13.64 ± 1.36	13.17 ± 1.32	17.43 ± 1.74	19.75 ± 1.98	16.59 ± 1.66	16.29 ± 1.63	15.19 ± 1.52	16.67 ± 1.67	22.35 ± 2.24	45.4 ± 4.54
3-hydroxy-5,6-epoxy-β-ionone (t.i.)	*2696*	-	6.26 ± 0.43	5.64 ± 0.39	3.21 ± 0.22	4.31 ± 0.3	11.31 ± 0.78	29.28 ± 2.02	26.15 ± 1.8	44.99 ± 3.1	13.17 ± 0.91	25.59 ± 1.77	11.17 ± 0.77	141.1 ± 9.74
3-hydroxy-7,8-dihydro-β-ionol	*2739*	*2722*	23.52 ± 2.12	21.18 ± 1.91	22.26 ± 2	24.2 ± 2.18	27.05 ± 2.43	27.76 ± 2.5	25.49 ± 2.29	21.46 ± 1.93	24.45 ± 2.2	27.93 ± 2.51	38.65 ± 3.48	40.4 ± 3.64
4-oxo-β-isodamascol (t.i.)	*2856*	-	13.87 ± 0.94	11.42 ± 0.78	10.96 ± 0.75	12.93 ± 0.88	14.77 ± 1	13.25 ± 0.9	12.18 ± 0.83	9.01 ± 0.61	12.04 ± 0.82	16.24 ± 1.1	29.86 ± 2.03	20.89 ± 1.42
benzenoids														
benzyl alcohol*	*1863*	*1876*	1.29 ± 0.11	0.98 ± 0.09	0.88 ± 0.08	2.15 ± 0.19	1.33 ± 0.12	1.3 ± 0.12	0.85 ± 0.08	1.34 ± 0.12	1.18 ± 0.11	1.1 ± 0.1	1.08 ± 0.1	1.19 ± 0.11
phenylethanol*	*1895*	*1893*	0.24 ± 0.02	0.22 ± 0.02	0.21 ± 0.02	0.25 ± 0.02	0.32 ± 0.03	0.29 ± 0.03	0.3 ± 0.03	0.25 ± 0.02	0.26 ± 0.02	0.3 ± 0.03	0.49 ± 0.04	0.43 ± 0.04
2-methoxy benzyl alcohol	*2138*	*2082*	7.65 ± 0.92	6.62 ± 0.79	7.95 ± 0.95	8.87 ± 1.06	7.96 ± 0.96	9.61 ± 1.15	9.9 ± 1.19	6.63 ± 0.8	7.53 ± 0.9	8.7 ± 1.04	9.66 ± 1.16	10.37 ± 1.24
α-cumyl alcohol	*1750*	*1779*	4.96 ± 0.3	5.11 ± 0.31	15.94 ± 0.96	15.58 ± 0.93	12.86 ± 0.77	17.47 ± 1.05	18.03 ± 1.08	4.35 ± 0.26	5.6 ± 0.34	5.6 ± 0.34	4.38 ± 0.26	15.2 ± 0.91
vanillic alcohol	*2778*	*2787*	16.05 ± 1.28	9.29 ± 0.74	10.88 ± 0.87	7.97 ± 0.64	8.65 ± 0.69	11.12 ± 0.89	10.4 ± 0.83	8.94 ± 0.72	14.71 ± 1.18	15.64 ± 1.25	21.32 ± 1.71	17.93 ± 1.43
methyl salicylate*	*1754*	*1759*	0.47 ± 0.04	0.33 ± 0.03	0.34 ± 0.03	0.49 ± 0.04	0.41 ± 0.04	0.27 ± 0.02	0.24 ± 0.02	0.29 ± 0.03	0.39 ± 0.04	0.35 ± 0.03	0.25 ± 0.02	0.21 ± 0.02

Three different areas (Le Moie, Massaccio, San Sisto), three harvest time (San Sisto H1, H2, H3), low and high vigour, three different clones and two different vinifications (late harvest and whitering process, skin maceration) volatile compounds in 2016 Verdicchio wines

TABLE 3. Odoriferous zones perceived by GC-AEDA.

RI wax^{exp}	RI ^{lit}	FD	Descriptor	Impact compound	CAS
1006	958	243	Apple	ethyl 2-methylpropanoate	97-62-1
1016	938	27	Kiwi	ethyl propanoate	105-37-3
1035	1041	81	Bilberry	ethyl 3-methylbutanoate	108-64-5
1024	1110	81	Banana	isoamyl acetate	123-92-2
1094	-	81	Toasted almonds	unknown	-
1080	1033	9	Apple/pineapple	ethyl butanoate	105-54-4
1201	1118	3	Herbaceous	isobutanol	78-83-1
1229	-	3	Balsamic	unknown	-
1179	1174	27	Harsh, stale	isoamyl alcohol	123-51-3
1225	1232	243	Bilberry/fruity	ethyl hexanoate	106-32-1
1264	1269	9	Fruity	hexyl acetate	142-92-7
1304	1302	27	Mushroom	1-octen-3-one	4312-99-6
1348	1345	3	Yeasty-creamy	unknown	-
1376	1328	3	Fruity	unknown	-
1437	1434	27	Fruity/apple	ethyl octanoate	106-32-1
1443	1454	27	Boiled potato	methional	3268-49-3
1466	1447	3	Vinegar	acetic acid	79-09-4
1523	1532	9	Fruity	ethyl 3-hydroxybutanoate	5405-41-4
1586	1570	27	Cheese, dog	isobutyric acid	503-74-2
1624	1635	3	Creamy, oily	ethyl furoate (t.i.)	614-99-3
1646	1620	27	Cheese	butyric acid	107-92-6
1634	1637	3	Fruity/sweaty	ethyl decanoate	110-38-3
1686	1671	81	Taleggio cheese	isovaleric acid	503-74-2
1730	1730	27	Boiled potato	3-methylthio-1-propanol	505-10-2
1732	1747	3	Aniseed	3-methyl-2,4-nonanedione	113486-29-6
1740	1730	9	Not defined	methyl salicylate	119-36-8
1739	1743	9	Gasoline	TDN	30364-38-6
1815	1838	243	Baked apple	β-damascenone	23726-93-4
1815	1810	9	Flowery/rose	phenethyl acetate/ethyl-4-hydroxybutanoate	103-45-7
1845	1875	3	Tropical/unpleasant	1-hexanol, 3-mercapto	51755-83-0
1864	1855	3	Pungent/chemical	hexanoic acid	142-62
1881	1875	9	Floral	benzenmethanol	100-51-6
1919	1909	243	Rose	2-phenylethanol	60-12-8
1962	1951	3	Sweet/green	unknown	-
1956	1939	81	Coconut	whiskey lactone I	39212-23-2
1960	1920	81	Violet	β-ionone	14901-07-6
2003	-	27	Licorice/vegetal	2H-pyran-2,6(3H)-dione (t.i.)	5926-95-4
2045	2047	243	Cotton candy	furaneol	3658-77-3
2050	2025	9	Acetic/bad	octanoic acid	124-07-2
2093	2080	243	Cotton candy	homofuraneol	27538-09-6
2044	-	9	Mushroom	unknown	-
2098	2123	27	Peach/sweet	γ-decalactone	706-14-9
2137	2168	9	Smoke-roast	ethyl 5-oxotetrahydro-2-furancarboxylate (t.i.)	1126-51-8
2196	2186	27	Leather/weiss beer	4-vinylguaiacol	7786-61-0
2200	2202	243	Celery like/sweet	sotolone	5579-78-2
2559	2556	243	Pastries, white chocolate	vanillin	121-33-5
2571	2555	27	Withered flower/honey	phenylacetic acid	103-82-2
2631	2640	27	Wood /clove	acetovanillone	498-02-2

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of some sulphur compounds and some carbonyl compounds, such as 3-MND. In such cases, it is necessary to use specific tests for the quantification of trace compounds. In order to quantify these compounds in our samples we used a derivatisation method with headspace solid phase microextraction using GC- tandem mass spectrometry. The derivatisation reagent PFBHA reacts with the carbonyl group formed oximes, which showed relatively specific mass spectra and high sensitivity. This method has been extensively employed to identify and quantify aroma components in several matrices, including wine (Flamini *et al.*, 2005) and beverages (López-Vázquez *et al.*, 2012; Saison *et al.*, 2009). Our method was modified from that of Moreira *et al.* (2013) and Saison *et al.* (2009), where the determination of carbonyl compounds in beer was reported. Table 1 shows the content of 3-MND in our samples.

As Verdicchio is not an aromatic grape, one of the special characteristics of this variety that has been shown in previous studies (Versini *et al.*, 2005) is the presence of a large amount of methyl salicylate. Methyl salicylate was found in *V. riparia* grapes (Schreier and Paroschy, 1980), in *V. vinifera* sp. (Cabaroglu *et al.*, 1997) and in the Frontenac interspecific hybrid (Mansfield *et al.*, 2011). The sensory impact of this compound in wine is not altogether clear. In the literature it is described as having an odour of wintergreen, mint and a fresh green character (Mansfield *et al.*, 2011). We found that in the free forms the concentration was in the range 25–40 µg L^{-1}, while in the bound forms, after hydrolysis, a higher concentration of up to 0.5 mg L^{-1} was found (Table 2). As the olfactory threshold of this compound is 50–100 µg L^{-1}, it is possible that in some Verdicchio wines the balsamic note of methyl salicylate contributes to the fresh/anise scent. It is well known that wine odours are complex mixtures of many volatile chemicals, present in different concentrations. These chemicals can interact synergistically or additionally in the mixtures according to unpredictable rules and are the basis of the overall odour sensation (Brattoli *et al.*, 2013). A recent study (Parker *et al.*, 2017) proposed the sensory role of monoterpene glycosides during tasting through retronasal perception of odorant aglycones released in-mouth. For this reason, studies are underway to quantify the precursors of methyl salicylate in wines, hence the total quantity, not only that deriving from hydrolysis of glycosylated compounds. In the literature it has been suggested that it can be bound as methyl salicylate 2-O-β-D-glucoside or as methyl salicylate 2-O-β-D-xylopyra-nosyl (1–6)–D-glucopyranoside (primeveroside), although nine different glycosides have been reported in plants to date (Mao *et al.*, 2014).

CONCLUSION

This work confirms that it is possible to obtain wines with very different characteristics from this variety of grapes, producing premium wines with a distinctive pattern of volatiles, reproducible across several vintages and variable depending on the different location of the vineyard and winemaking techniques. Young wines are characterised by fruity, thiolic notes, while wines aged for longer are distinguished by their norisoprenoid content and by balsamic, anise and liquorice notes, which can be attributed to methyl salicylate released by precursors and the presence of 3-MND and pantolactone. With a new derivatisation and GC-MS-MS method it was possible to quantify 3-MND in the range10–50 ng L^{-1}. With the use of GC-O, 48 sensorially important compounds were found for this wine. Analysis of the compounds after hydrolysis confirmed that a high amount of methyl salicylate characterised this variety. Methyl salicylate has a balsamic note from wintergreen oil that is often perceived in Verdicchio tasting. In-depth studies are underway to understand which precursors are present in grapes and whether they are in any way dependent on agro-climatic variables.

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