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Original article

Global volatile signature and polyphenols patterns in Vespolina wines according to vintage

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Summary The global volatile signature of Vespolina wines from different vintages was established using solid-phase microextraction combined with gas chromatography–mass spectrometry (HS-SPME/GC-qMS). Wines were also characterised in terms of bioactive compounds (such as individual polyphenols, biogenic amines and their precursors) by high-performance liquid chromatography (RP-HPLC). In addition, some physicochemical parameters, such as the total phenolic content, total tannins and antioxidant capacity, were evaluated. Seventy-one volatile compounds and thirty-three bioactive compounds were identified in Vespolina wines. The application of multivariate analysis to the obtained data revealed that 2-phenylethyl acetate, ethyl nonanoate, 2-hexanol, isoamyl octanoate and ethyl 2-hydroxymethylbutanoate were the primary compounds responsible for Vespolina wines classification, mainly indicative for wines of 2015 and 2013 vintages. Conversely, wines from 2008 and 2009 vintages showed highest values of procyanidin B1, catechin, gallic acid, *trans*-piceid and *trans*-resveratrol.

Keywords HS-SPME/GC-qMS, Phenolic compounds, Vespolina, Volatile compounds.

Introduction

Vespolina, also called Ughetta di Canneto, is a red grape cultivar that produces wines characterised by floral, spicy and peppery notes (Caputi et al., 2011; Mattivi *et al*., 2011). Vespolina is an autochthonous cultivar to northwestern Italy, mainly located in the Piedmont and Lombardy regions. The variety is moderately vigorous, showing a good basal fertility. Vespolina produces characteristic dark-red wines (DOC Colline Novaresi Vespolina, DOC Coste della Sesia Vespolina) typically characterised by particularly intense spicy notes. Wines' chemical fingerprint is characterised by volatile and non-volatile secondary metabolites, which are responsible for their sensorial and nutritional properties (Perestrelo et al., 2008). Regarding the volatile signature, some volatile organic compounds (VOCs) are biosynthesised during ripening and therefore are present in grapes (*e.g*. terpenoids and C_{13} norisoprenoids); however, most of them are formed during the process of fermentation (e.g. esters, alcohols, acids, carbonyl compounds) by yeasts (commonly *Saccharomyces cerevisiae* strains) and subsequently during the wine storage (e.g. lactones, volatile phenols, furanic compounds) (Sagratini *et al*., 2012; Ivanova *et al*., 2013). Currently, many VOCs belonging to different chemical families have been identified in grapes and wines at levels ranging from ng L^{-1} to mg L^{-1}. The VOCs levels in wines can be affected by agronomic (*e.g*. grape variety, ripening stage, edaphoclimatic condition) and technological (*e.g*. harvest, storage and processing conditions) factors (Sagratini *et al*., 2012; Andreu-Sevilla, *et al*., 2013).

Several studies have been performed combining headspace solid-phase microextraction (HS-SPME) with gas chromatography quadrupole mass

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spectrometry (GC-qMS) in order to characterise the volatile profile of different food matrices, such as fruits (Ferreira *et al*., 2009), grapes (Perestrelo et al., 2008), wines (Andreu-Sevilla *et al*., 2013; Ivanova *et al*., 2013), among others.

SPME offers several advantages like simplicity, high sensitivity and reproducibility over traditional techniques (e.g. solid-phase extraction and liquid–liquid extraction (Silva *et al*., 2014). In parallel, several methods employing high-performance liquid chromatography (HPLC) have been developed because of the complexity of the phenolic composition of wine. Since HPLC can detect in a single analysis different groups of phenolics, most studies have reported the identification of these classes of compounds in wine (Gómez-Alonso *et al*., 2007; Lorenzo *et al*., 2017). The phenolic compounds play a crucial role on the wine, responsible for quality and sensorial characteristics such as taste and colour. They also contribute to oxidation reactions and ageing. However, in excess can also negatively affect the wine aroma (Xing *et al*., 2016). Level of phenolic compounds in wines depends on grape variety, terroir, vineyard location, vine cultivation practices, ripeness, winemaking process and phenolic reactivity during winemaking and ageing (Pereira *et al*., 2013; Budić-Leto *et al*., 2017). Phenolic compounds have been shown to have antioxidant activities, and therefore, they are linked to reduced risk of oxidative stress mediated diseases (e.g. cancer, cardiovascular disease (Kivilompolo *et al*., 2008)). Despite the increasing production and consumer interest in Vespolina wines, remains a lack of scientific literature concerning to its chemical composition. Thus, and for the first time, we aim establish and compare the pattern of volatile and phenolic composition of red wines obtained from Vespolina grape variety from six vintages (2008, 2009, 2011, 2012, 2013 and 2015). Furthermore, wines were characterised through some parameters such as total tannins, phenolic and anthocyanin contents, and antioxidant activity. A statistical analysis, including principal components analysis (PCA) and hierarchical cluster analysis (HCA), was applied.

Material and methods

Reagents and material

All chemicals were analytical quality grade. Standards of volatile compounds used for identification of target compounds were purchased from Sigma-Aldrich (Madrid, Spain), Acros Organics (Geel, Belgium) and Fluka (Buchs, Switzerland) with purity > 98%. HPLC grade water was obtained from an ELGA PURELAB Ultra system (M-medical, Cornaredo, Milano, Italy). All the analytical standards (polyphenols, amino acids and biogenic amines) were purchased from Sigma-Aldrich (Milan, Italy).

Samples

Monovarietal Vespolina wines from vintages 2008, 2009, 2011, 2012, 2013 and 2015 were produced by the same vineyard located in the Northern part of Piedmont Region (Ghemme – Italy; 260 m a.s.l.). The wines were produced following the standard protocols used for the 'Colline Novaresi D.O.C. Vespolina' wine appellation (100% Vespolina cultivar). Table 1 reports the climatic data (average temperature and rainfall) of each vintage registered in the vineyard area together with some grapes parameters. Age of vineyard: 20 years (2009 vintage); southwest exposure. Alcoholic fermentation lasted an average period of 8 days; followed by malolactic fermentation, performed inside stainless steel tank, and chemical stabilisation (winter period). Finally, wines were bottled the spring following the vintage without wood ageing. Three bottles every vintage were sampled, aliquoted and stored at −20°C until analysis.

Physicochemical characterisation of Vespolina wines

Colour determination

The colour of the different Vespolina samples was detected by using the classic method described for wines (Glories, 1984). Briefly, the wine samples were diluted with distilled water (10-fold), and the absorbance (A) was recorded at 420, 520 and 620 nm by using a spectrophotometer (DU 530, Beckman Coulter, Milan, Italy). The colorimetric indices were calculated applying the following formulas: colour intensity ($CI = A_{420} + A_{520} + A_{620}$), colour tonality ($CT = A_{420}/A_{520}$), % yellow ($\%Y = A_{420}/CI*100$), % red ($\%R = A_{520}/CI*100$) and % blue ($\%B = A_{620}/CI*100$).

Total phenolic content

The estimation of total phenolic content (TPC) was performed by the Folin-Ciocalteu assay according to Singleton & Rossi (1965) within minor modification. The results were expressed as milligrams of gallic acid equivalent (GAE) per litre of sample. Absorbance at 760 nm was recorded (DU 530, Beckman Coulter, Milan, Italy) using 1 cm quartz cells.

Total tannins

The n-butanol/HCl method, previously reported by Bate-Smith (1973), was used to measure the content of condensed tannins in wine samples. Absorbance at 550 nm was recorded (DU 530, Beckman Coulter, Milan, Italy); the total tannin (TT) content was determined using 0.1736 as conversion factor.

Antioxidant capacity

The DPPH free radical scavenging method was performed following the procedure previously reported by

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Locatelli *et al.* (2009). Briefly, 700 μL of diluted (1:500 with MeOH) wine sample or MeOH (control) was added to the same volume of methanolic solution of DPPH• (100 μM). The antioxidant capacity of wines was expressed as inhibition percentage (I %) of the radical.

Anthocyanin content

Determination of anthocyanin content was performed in acidified ethanol as previously reported by Glories (1999). Wine samples were diluted (50-fold) with acidified (1% HCl) ethanol solution (70%). The absorbance was recorded at 540 nm (DU 530, Beckman Coulter, Milan, Italy). Total anthocyanin content (TA) was expressed as malvidin equivalents (ME).

HS-SPME and GC-qMS analysis

The HS-SPME experimental parameters were those optimised in a previous study (Perestrelo et al., 2008). A 50/30-μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre was used. Aliquots of 30 mL wine were placed into a 60-mL amber glass vial, followed by the addition of 30% (w/v) of NaCl and capped with a PTFE. The thermostatic bath temperature was 40.0 °C; conditioning and fibre exposure times were 5 and 45 min, respectively. Each wine sample was analysed in triplicate.

All chromatographic analyses were performed using an Agilent Technologies 6890N Network gas chromatograph system (Palo Alto, CA) interfaced with an Agilent 5975 quadrupole inert mass selective detector. A 60 m × 0.25 mm ID × 0.25 mm film thickness BP-20 (SGE, Dortmund, Germany) fused silica capillary column was used. Separation was performed starting at 45°C, held for 5 min, temperature increased at a rate of 2.5°C min^{-1} to 150 °C (held for 5 min) and finally increased 15°C min^{-1} to 220 °C (held for 20 min). Analysis was performed in the splitless mode at 1.0 mL min^{-1} constant flow; helium was used as carrier gas. The operating temperatures used (transfer line, quadrupole and ionisation source) were 220, 150 and 230°C, respectively. Ionisation voltage was set at 70 eV, and ionisation current was set at 10 μA. The identification of VOCs were based on comparison of their mass spectra with pure standards when available, and/or with those reported by the MainLib NIST05 commercial library with a similarity threshold higher than 80%. A series of C_8-C_{20} n-alkanes were analysed using the same methodology to establish the kovats retention indices (KI) and to confirm the identity of the VOCs by comparison with the literature (Chen *et al.*, 1982; Chung *et al.*, 1993; Nishimura, 1995; Ruther, 2000; Ferreira *et al.*, 2001; Wei *et al.*, 2001; Alves & Franco, 2003; Choi, 2003; Lee & Noble, 2003; Qian & Reineccius, 2003; Varming *et al.*, 2004; Spínola *et al.*, 2015).

Polyphenol analysis

The polyphenol analysis was performed according to the method reported by Bordiga et al. (2015). The chromatographic system used was a Shimadzu LC-20A Prominence with a diode array detector (SPD-M20A). The column used for the reversed-phase separation was a Synergi^{TM} (250 × 4.6 mm, with particle size of 4 μm; Phenomenex, Torrance, CA). Eluent A consisted of water/formic acid/acetonitrile (87:10:3 v/v) and eluent B water/formic acid/acetonitrile (40:10:50 v/v).

Determination of biogenic amines and precursors

Biogenic amines (histamine, tyramine, tryptamine and 2-phenylethylamine) and their precursors were detected following the procedure previously reported (Bordiga *et al.*, 2020). The chromatographic system used was a Shimadzu Class VP HPLC equipped with a UV–Vis detector SPD-10A. The column used was a Spherisorb S5 ODS2 (250 mm × 4.6 mm I.D., particle size 5 μm). Eluent A: aqueous solution (adjusted pH to 3.5 with phosphoric acid) with heptane sulphonate (8.3 mM), KH_2PO_4 (9.0 mM) and 20 μL L^{-1} of octylamine. Eluent B: Methanol.

Statistical analysis

Results were expressed as mean ± standard deviation (SD) of at least three independent experiments. Differences among samples were estimated by analysis of variance (ANOVA) followed by Tukey's 'honest significant difference' test. The statistical significance level was set to 0.05. The hierarchical cluster analysis (HCA) was performed using SIMCA-p 14.1 software (Umetrics AB, Umeå, Västerbotten, Sweden). The principal component analysis (PCA) was performed using IBM Statistical Package for the Social Sciences (SPSS) statistical software 19.0 (IBM, New York, NY, USA).

Results and discussion

Global volatile signature of Vespolina wines by HS-SPME/GC-qMS

Seventy-one VOCs were identified in Vespolina wine samples (Table S1, Supplementary Material). Moreover, Figures S1 and S2 show the total GC peak areas of each chemical family identified in Vespolina wines.

According to the results, the volatile profiles of Vespolina wines of all vintages are mainly composed of alcohols and esters, which contribute 95.42, 95.61, 65.46, 95.90, 95.66 and 95.70% for 2008, 2009, 2011, 2012, 2013 and 2015 vintages, respectively.

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The alcohol contributions, mainly expressed by 3-methylbutan-1-ol and phenylethyl alcohol, range from 52.92 to 56.83 %. These alcohols represents 87.74, 90.27, 89.06, 89.57, 90.67 and 89.52 % of total alcohols profile on 2008, 2009, 2011, 2012, 2013 and 2015 vintages, respectively. The presence of 3-methylbutan-1-ol and phenylethyl alcohol could contribute positively with floral and fruity notes. In qualitative terms, 2-ethylhexan-1-ol (citrus, fresh) and 2,3-butanediol isomers (green, buttery) were not detected in 2015 vintage, whereas 2-hexen-1-ol (floral) and 2-hexanol (green) were not detected in 2008 and 2012 vintages, respectively. In quantitative terms, for total GC peak area, Vespolina wines from 2011 and 2015 vintages showed the lowest alcohol content.

In wine, one of the most important chemical families of aroma compounds is ethyl esters (mainly with 6-10 carbon atoms), which positively influence their fruity and floral notes. This chemical family contributes for 42.50, 41.31, 39.63, 40.30, 39.71 and 41.01 % of the total volatile profile on 2008, 2009, 2011, 2012, 2013 and 2015 vintages, respectively. The major ethyl esters found in Vespolina wines were ethyl acetate, ethyl octanoate, ethyl decanoate and diethyl succinate, which can contribute positively to aroma with floral, fruity, sweet and wine-like notes. These four ethyl esters represent 81.24, 84.63, 83.79, 82.93, 56.95 and 85.42 % of total esters profile for 2008, 2009, 2011, 2012, 2013 and 2015 vintages, respectively. Data obtained in relation to ethyl esters were in broad agreement with those previously described in the literature for monovarietal wines produced with well-known red grape varieties (Cabernet Sauvignon, Merlot, Syrah) (González-Centeno et al., 2019). Moreover, all Vespolina wines showed a similar pattern, with exception of 2015 vintage where ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, ethyl 2-hexenoate, ethyl 2-hydroxymethylbutanoate and diethyl glutarate were not detected. Ethyl 2-hexenoate (rum-like odour) was not detected in 2013 vintage.

The acids contribution to total volatile profile range from 2.42% to 3.76 %. Acetic and hexanoic acids were the predominant acids found in Vespolina wines, independently of vintage, representing on average 72.34 % of total acids profile. In qualitative terms, no significant differences ($P < 0.05$) were observed among all vintages, and in semi-quantitative terms, the Vespolina wines from 2011 showed the lowest content of this chemical family.

Linalool, β-terpineol and guaiazulene are the most abundant terpenoids found in Vespolina wines, which represent 50.35, 61.34, 56.07, 57.04, 58.06 and 45.51 % of total terpenoids profile for 2008, 2009, 2011, 2012, 2013 and 2015, respectively. Clearly, even if characterised by a very low threshold, terpenoids have a more significant impact in aromatic white wines (e.g. Muscat) (Bordiga *et al.*, 2013).

Vitispirane I and II are the predominant C_{13} norisoprenoids found in Vespolina wines, representing on average 82.05 % of total of C_{13} norisoprenoids.

Regarding other chemical groups identified, butyrolactone contributes 0.10, 0.08, 0.10, 0.07, 0.06 and 0.04% to total volatile profile for 2008, 2009, 2011, 2012, 2013 and 2015 vintages, respectively. This lactone could contribute positively to aroma profile with caramel, coconut, cream and peach notes (Bordiga *et al.*, 2014).

Physicochemical characterisation of Vespolina wines

The pH value represents an important parameter, since it is also related to the formation of biogenic amines (Martuscelli *et al.*, 2013). In Vespolina wines, the pH ranged from 3.41 to 3.64, in accordance with other red wines (3.2 - 3.7) (Locatelli *et al.*, 2016) (Table S2).

Vespolina of the 2011 vintage showed the highest TPC (2789 mg L^{-1}), followed by the 2012, 2008, 2013, 2015 and 2009 vintages. Antioxidant capacity showed a decreasing trend following this order: 2011, 2008, 2012, 2013, 2009 and 2015 vintages. On average, the Vespolina wines from the last two vintages (about 37-39 % inhibition) showed an AC one and a half times lower when compared to wines from 2011 (53 % inhibition). Total anthocyanin content (TA) ranged from about 180 mg L^{-1} malvidin eq. (2015 vintage) to nearly 100 mg L^{-1} malvidin eq. (2008 vintage). On average, the TT levels of Vespolina wines ranged from about 6 g L^{-1} for 2011 vintage to nearly 4 g L^{-1} for 2015 vintage.

Polyphenol profiles of Vespolina wines

Vespolina wines from 2015 showed the highest values of anthocyanins, conversely to the 2008 and 2009 that represented the lowest levels (Table 2). Peonidin-3-O-glucoside and malvidin-3-O-glucoside are the most abundant compounds of this class ranging from 20.5 to 0.53 mg L^{-1} and 10.9 to 0.01 mg L^{-1}, respectively.

Caftaric and coutaric acids are the most abundant, while sinapic and ferulic acids were detected in trace amounts (0.70-0.20 and 0.89-0.54 mg L^{-1}, respectively), in agreement with previous data reported in literature (Puértolas *et al.*, 2011; Bimpilas *et al.*, 2015).

The 2012 and 2008 vintages showed the highest flavan-3-ols concentration (244.6 and 242.4 mg L^{-1}) followed by the 2013, 2011, 2015 and 2009 vintages, with 209.6, 172.1, 148.1 and 91.8 mg L^{-1}, respectively. Although similar as regards the total higher values, the two vintages show significant differences in

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qualitative terms. The highest concentration of procyanidin B2 was detected in 2008 vintage, the lowest in 2009 wines (ranging from about 26 to 10 mg L^{-1}, respectively), in agreement with previous studies (Chira *et al.*, 2011; Silva *et al.*, 2012). Catechin ranged from 31 to 79 mg L^{-1}, whereas epicatechin ranged from 9 to 24 mg L^{-1}.

Among flavonols, quercetin-3-O-galactoside and kaempferol-3-O-rutinoside were the most abundant in all vintages. Myricetin ranged from 1.62 to 2.45 mg L^{-1}, whereas quercetin from 1.85 to 2.18 mg L^{-1}.

Among stilbenes, an important class of compounds thanks to their bioactivity (Ribeiro de Lima *et al.*, 1999), trans- and cis-resveratrol and related piceids were detected in all vintages (Table 3). The concentration of trans-resveratrol in Vespolina wines ranged from 1.15 (2008) to 0.42 (2012) mg L^{-1}, whereas cis-resveratrol ranged from 1.94 (2015) to 0.56 (2008) mg L^{-1}. The content of trans-piceid ranged from 5.36 (2009) to 3.80 (2012) mg L^{-1}, whereas cis-piceid level ranged from 8.85 (2015) to 4.42 (2008) mg L^{-1}, in agreement with previously reported data (Moreno-Labanda *et al.*, 2004).

Table 1 Climatic data (average temperature and rainfall) of each vintage registered in the vineyard area together with some grapes parameters

Harvest	2008 9 Oct	2009 24 Set	2011 20 Set	2012 22 Set	2013 3 Oct	2015 18 Set
°Babo	20.5	20.5	21.5	20	19	18.5
Yield (q)	80	90	36*	63	58	102
% Ethanol (v/v)	12.7	12.8	13.1	12.5	12.3	12.0
Avg Temp. (°C)						
January	3.5	0.7	0.8	2.1	3.0	3.7
February	4.5	3.7	4.6	0.7	1.9	4.0
March	8.3	8.6	7.8	11.3	5.6	8.8
April	10.7	12.4	15.1	10.6	11.7	12.8
May	15.8	18.4	18.0	16.1	14.1	16.9
June	19.9	20.3	19.4	20.7	19.9	20.9
July	21.5	22.3	20.3	22.5	23.1	25.7
August	21.3	23.2	22.8	23.3	21.7	22.2
September	16.2	18.4	19.7	17.6	17.9	16.9
Rainfall (mm)						
January	72.0	52.6	23.2	35.8	19.8	66.6
February	39.6	119.2	61.0	10.8	36.4	134.4
March	40.0	149.4	144.2	56.0	112.8	75.2
April	169.2	280.0	33.4	201.0	160.2	142.4
May	191.8	18.0	76.2	171.0	169.2	88.2
June	128.2	69.4	151.0	88.4	24.4	10.8
July	96.2	67.8	57.4	63.4	98.6	14.2
August	57.8	47.4	12.0	37.8	57.2	160
September	72.4	100.0	62.8	64.4	82.0	98.2
Total	867.2	903.8	621.2	728.6	760.6	790.0

*Hailstorm in May (data obtained from ARPA Piemonte – Agenzia Regionale per la Protezione Ambientale del Piemonte).

Biogenic amines and precursors

On average, histamine presents the highest level (range 5.6-11.2 mg L^{-1}), followed by phenylethylamine (2.3-4.5 mg L^{-1}), tyramine (0.17-1.4 mg L^{-1}) and tryptamine (0.02-0.9 mg L^{-1}) (Table 4). The sum of biogenic amines in 2008 and 2013 vintages appeared to be similar (14.7 and 14.6 mg L^{-1}). Vespolina wines of 2015 vintage showed the lowest value, reaching 9.1 mg L^{-1}. Histamine reached the highest values in 2013 wines (about 11 mg L^{-1}) while tryptamine was highest in 2008 wines (about 0.9 mg L^{-1}).

Multivariate statistical data analysis

Environmental factors (topographical, agro-pedological, climatic) have been acknowledged to influence grape and wine volatiles and non-volatiles composition. For Vespolina, the grape harvest usually takes place during the third week of September, however, in 2008 and 2013 vintages, and this was done with almost 15 days late. Average temperatures and rainfall showed differences in the six vintages that have clearly influenced the composition of the grapes (Table 1).

Looking at the GC data, Figure 1 shows the PCA score plot, together with the loading weight plot, of the two first principal components (PC1 vs PC2), which explains 67.3% of the total variability of the data set. The 2011 vintage was singular, characterised by the lowest total rainfall (621 mm) and the highest average temperature in April. Grape yield was also the lowest due to a hailstorm during the month of May. Samples from 2011, located in negative PC2, showed higher values of ethyl heptanoate and ethyl 2-hexenoate and the lowest value of 3-octanone (Figure S3).

Wines from 2015 vintage were characterised by higher values of hexyl acetate, 2-phenylethyl acetate and ethyl nonanoate. The 2015 vintage had an average temperature in the month of July of almost 26°C. Total rainfall was about average but distributed abnormally, a very rainy February but it touched historical lows in June and July. Wines from 2013 vintage (late harvest) showed the highest values of 2-hexanol, isoamyl octanoate and ethyl 2-hydroxymethylbutanoate. In 2013, avg temperature of May was about two degree lower compared to the others whereas spring was rainy with a low for the month of June. Wine from 2008 vintage was characterised by highest values of methyl octanoate, 3-methyl-1-butanol and o-Cymene. Wine from 2009 vintage showed the highest content of 3-methyl-1-pentanol and ethyl 3-methylbutanoate. Wine from 2012 vintage was characterised by the lowest values of 1,2-dihydro-1,1,6-trimethylnaphthalene (DTN) and high content of methyl 2-hydroxybenzoate.

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Table 2 Concentration (mg L^{-1}) of polyphenols identified in Vespolina wines from different vintages

mg L^{-1}	detection wavelength (nm)	2008	2009	2011	2012	2013	2015
Anthocyanins							
Delphinidin-3-O-glucoside	520	0.15 ± 0.001^{D}	0.06 ± 0.002^{D}	0.06 ± 0.002^{D}	0.45 ± 0.006^{C}	2.63 ± 0.024^{B}	4.24 ± 0.022^{A}
Cyanidin-3-O-glucoside	520	0.14 ± 0.004^{D}	0.17 ± 0.002^{D}	0.17 ± 0.007^{D}	0.39 ± 0.002^{C}	1.05 ± 0.023^{B}	1.84 ± 0.029^{A}
Petunidin-3-O-glucoside	520	0.20 ± 0.003^{D}	0.12 ± 0.001^{E}	0.12 ± 0.002^{E}	0.63 ± 0.020^{C}	2.66 ± 0.018^{B}	5.28 ± 0.028^{A}
Peonidin-3-O-glucoside	520	1.10 ± 0.013^{D}	0.53 ± 0.003^{E}	1.14 ± 0.026^{D}	2.86 ± 0.016^{C}	9.45 ± 0.029^{B}	20.5 ± 0.054^{A}
Malvidin-3-O-glucoside	520	0.11 ± 0.004^{D}	0.01 ± 0.003^{E}	0.13 ± 0.005^{D}	0.75 ± 0.007^{C}	3.23 ± 0.015^{B}	10.9 ± 0.012^{A}
Hydroxybenzoic/Hydroxycinnamic acids							
Gallic acid	280	48.3 ± 0.044^{A}	26.4 ± 0.045^{BC}	31.4 ± 0.047^{B}	23.9 ± 0.054^{C}	32.1 ± 0.091^{B}	11.7 ± 0.038^{D}
Protocatechuic acid	280	6.47 ± 0.030^{A}	5.50 ± 0.052^{AB}	3.98 ± 0.060^{C}	5.78 ± 0.041^{A}	5.09 ± 0.023^{B}	4.28 ± 0.016^{C}
Caffeic acid	330	10.6 ± 0.015^{B}	3.89 ± 0.013^{C}	1.68 ± 0.012^{D}	20.1 ± 0.033^{A}	11.0 ± 0.058^{B}	2.77 ± 0.021^{C}
p-Coumaric acid	330	6.77 ± 0.038^{B}	1.62 ± 0.050^{C}	1.13 ± 0.018^{C}	11.3 ± 0.056^{A}	5.64 ± 0.024^{B}	nd
Ferulic acid	330	0.81 ± 0.017^{A}	0.74 ± 0.024^{B}	0.72 ± 0.025^{B}	0.89 ± 0.010^{A}	0.68 ± 0.015^{B}	0.54 ± 0.009^{C}
Sinapic acid	330	0.40 ± 0.002^{B}	0.30 ± 0.001^{BC}	0.70 ± 0.003^{A}	0.22 ± 0.002^{C}	0.20 ± 0.003^{C}	0.21 ± 0.001^{C}
Flavan-3-ols							
Procyanidin B1	280	66.7 ± 0.237^{A}	21.6 ± 0.007^{D}	51.6 ± 0.072^{B}	54.8 ± 0.254^{B}	54.9 ± 0.168^{B}	39.5 ± 0.091^{C}
Catechin	280	78.8 ± 0.105^{A}	31.3 ± 0.183^{C}	59.8 ± 0.272^{B}	59.1 ± 0.164^{B}	59.9 ± 0.113^{B}	53.6 ± 0.200^{BC}
Procyanidin B2	280	25.6 ± 0.152^{A}	10.6 ± 0.105^{C}	22.1 ± 0.102^{B}	20.4 ± 0.283^{B}	24.5 ± 0.122^{A}	14.2 ± 0.162^{C}
Epigallocatechin gallate	280	2.22 ± 0.024^{B}	1.91 ± 0.027^{B}	1.33 ± 0.031^{C}	2.54 ± 0.023^{AB}	3.32 ± 0.142^{A}	1.55 ± 0.041^{C}
Epicatechin	280	23.7 ± 0.219^{A}	9.42 ± 0.234^{C}	20.3 ± 0.201^{AB}	17.1 ± 0.094^{B}	18.2 ± 0.111^{B}	22.9 ± 0.333^{A}
Gallocatechin gallate	280	35.4 ± 0.336^{B}	10.8 ± 0.049^{C}	3.43 ± 0.138^{D}	73.9 ± 0.100^{A}	33.9 ± 0.205^{B}	5.75 ± 0.062^{D}
Epicatechin gallate	280	4.21 ± 0.132^{AB}	3.26 ± 0.044^{B}	2.54 ± 0.055^{C}	1.51 ± 0.023^{D}	3.53 ± 0.069^{B}	5.00 ± 0.086^{A}
Procyanidin A2	280	5.18 ± 0.104^{C}	4.10 ± 0.128^{CD}	13.2 ± 0.066^{A}	14.9 ± 0.062^{A}	10.9 ± 0.096^{B}	3.44 ± 0.119^{D}
Flavonols							
Quercetin-3-O-galactoside	330	6.33 ± 0.059^{A}	5.72 ± 0.043^{B}	5.73 ± 0.038^{B}	5.16 ± 0.044^{B}	2.65 ± 0.023^{C}	6.09 ± 0.022^{A}
Quercetin-3-O-glucoside	330	1.38 ± 0.012^{C}	0.94 ± 0.019^{D}	1.39 ± 0.007^{C}	1.71 ± 0.021^{B}	1.70 ± 0.025^{B}	2.78 ± 0.053^{A}
Kaempferol-3-O-rutinoside	330	4.77 ± 0.039^{B}	6.73 ± 0.078^{A}	1.24 ± 0.024^{E}	2.41 ± 0.046^{D}	3.23 ± 0.063^{C}	1.36 ± 0.033^{E}
Kaempferol-7-O-neohesperidoside	330	0.31 ± 0.004^{A}	nd	0.30 ± 0.029^{A}	0.23 ± 0.010^{B}	0.21 ± 0.009^{B}	nd
Kaempferol-3-O-glucoside	330	3.14 ± 0.062^{A}	1.89 ± 0.013^{C}	2.58 ± 0.028^{B}	2.49 ± 0.015^{B}	2.65 ± 0.035^{B}	2.10 ± 0.011^{BC}
Myricetin	330	1.62 ± 0.005^{C}	1.77 ± 0.012^{C}	1.99 ± 0.003^{B}	1.97 ± 0.005^{B}	2.45 ± 0.032^{A}	2.27 ± 0.006^{A}
Quercetin	330	2.18 ± 0.010^{A}	1.94 ± 0.006^{AB}	2.10 ± 0.005^{A}	2.00 ± 0.013^{AB}	1.89 ± 0.008^{B}	1.85 ± 0.005^{B}
Kaempferol	330	1.51 ± 0.009^{AB}	1.26 ± 0.007^{B}	1.81 ± 0.036^{A}	1.50 ± 0.003^{AB}	1.18 ± 0.005^{B}	1.14 ± 0.006^{B}
Tartaric esters of hydroxycinnamic acids							
Caftaric acid	280	47.8 ± 0.024^{B}	55.1 ± 0.029^{B}	7.05 ± 0.022^{E}	21.3 ± 0.012^{D}	35.6 ± 0.045^{C}	89.5 ± 0.284^{A}
Cutaric acid	280	28.5 ± 0.077^{B}	30.1 ± 0.028^{B}	4.89 ± 0.021^{E}	10.7 ± 0.027^{D}	18.8 ± 0.024^{C}	38.6 ± 0.041^{A}

Values with different lowercase letters within the same row are significantly different ($P < 0.05$).

Looking at the fifty-one analytical variables, the two first principal components (PC1 vs PC2) explain 62.8 % of the total variability of the data set (Figure 1).

Wine from 2009 vintage showed higher values of kaempferol-3-O-rutinoside, *trans*-piceid and quercetin-3-O-galactoside. The 2009 vintage showed the highest total rainfall (903 mm), showing a maximum in April and September. Similarly, the 2008 vintage was characterised by a rainy spring and normal temperatures. Wine of this vintage showed the highest values of gallic acid, tryptamine and trans-resveratrol.

Wine from 2015 vintage, located in negative PC1, showed highest values of anthocyanins. High values of myricetin, epicatechin and epicatechin gallate have been detected in wine from 2013 vintage. Wine from 2011 vintage showed the highest content of sinapic acid, tyramine and kaempferol. Highest content of caffeic acid, gallocatechin gallate and procyanidin A2 has been reported in wine from 2012 vintage.

The overall plant responses towards the different environmental factors are evident in the vintages. Defence response mechanisms involve specific modification in the state of metabolic gene expression networks, which affect protein synthesis to modulate associated primary and secondary metabolite pathways. Most of the variations in the contents of the detected compounds are due, mainly, to the impact of environmental differences among the years mainly in terms of temperature, light, humidity and rainfall. These environmental factors will obviously impact the physiology of the plant influencing some metabolic pathways and therefore the biosynthesis of aromatic

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Table 3 Concentration (mg L^{-1}) of stilbenes in Vespolina wines from different vintages

Vintage mg L^{-1}	trans-Piceid	trans-Resveratrol	cis-Piceid	cis-Resveratrol
2008	5.26 ± 0.021^{A}	1.15 ± 0.013^{A}	4.42 ± 0.053^{C}	0.56 ± 0.012^{D}
2009	5.36 ± 0.050^{A}	0.61 ± 0.007^{C}	7.62 ± 0.020^{AB}	1.06 ± 0.006^{C}
2011	4.58 ± 0.017^{B}	0.77 ± 0.015^{B}	8.84 ± 0.067^{A}	1.64 ± 0.008^{B}
2012	3.80 ± 0.006^{C}	0.42 ± 0.002^{D}	6.41 ± 0.079^{B}	1.09 ± 0.006^{C}
2013	3.89 ± 0.031^{C}	0.62 ± 0.006^{C}	6.42 ± 0.037^{B}	1.18 ± 0.017^{C}
2015	4.33 ± 0.100^{B}	0.74 ± 0.006^{B}	8.85 ± 0.060^{A}	1.94 ± 0.018^{A}

Values with different lowercase letters within the same column are significantly different ($P < 0.05$).

Table 4 Concentration (mg L^{-1}) of biogenic amines and their amino acids precursors in Vespolina wines from different vintages

Vintage mg L^{-1}	Tyrosine	Histidine	Phenylalanine	Tryptophan	Tyramine	Histamine	Phenylethylamine	Tryptamine
2008	60.9 ± 0.042^{A}	194 ± 0.117^{A}	254 ± 0.312^{A}	7.82 ± 0.027^{A}	0.66 ± 0.017^{D}	9.96 ± 0.097^{AB}	3.16 ± 0.123^{B}	0.92 ± 0.011^{A}
2009	19.4 ± 0.089^{D}	76.3 ± 0.062^{D}	90.9 ± 0.125^{D}	1.26 ± 0.013^{D}	1.22 ± 0.07^{AB}	6.98 ± 0.128^{C}	3.45 ± 0.114^{B}	0.37 ± 0.012^{B}
2011	52.3 ± 0.070^{B}	168 ± 0.152^{B}	241 ± 0.523^{A}	4.03 ± 0.016^{B}	1.44 ± 0.072^{A}	6.97 ± 0.083^{C}	4.54 ± 0.117^{A}	0.32 ± 0.009^{B}
2012	49.5 ± 0.095^{B}	175 ± 0.292^{B}	189 ± 0.864^{B}	7.75 ± 0.015^{A}	0.85 ± 0.006^{C}	8.34 ± 0.157^{B}	2.44 ± 0.080^{C}	0.17 ± 0.001^{C}
2013	50.9 ± 0.024^{B}	195 ± 0.884^{A}	184 ± 0.650^{B}	4.37 ± 0.008^{B}	1.08 ± 0.053^{B}	11.2 ± 0.039^{A}	2.29 ± 0.053^{C}	0.02 ± 0.001^{D}
2015	33.2 ± 0.151^{C}	119 ± 0.314^{C}	162 ± 0.230^{C}	2.08 ± 0.022^{C}	0.17 ± 0.004^{E}	5.61 ± 0.119^{D}	3.27 ± 0.212^{B}	0.05 ± 0.002^{D}

Values with different lowercase letters within the same column are significantly different ($P < 0.05$).

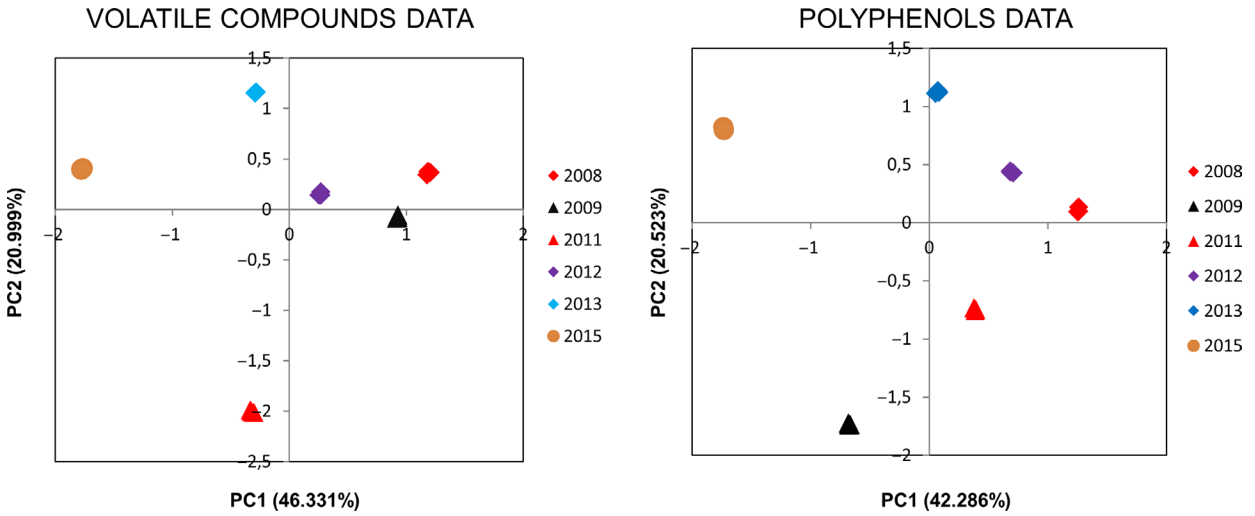

Figure 1 PCA score scatter plots obtained by using volatile compounds (left) and polyphenols (right) data ($n = 3$ for each data point). The PCA models were obtained with two first principal components that explained 67.330% and 62.809% of the total response variance, respectively. [Colour figure can be viewed at wileyonlinelibrary.com]

compounds and their precursors namely those associated with varietal aroma of grapes (C6 alcohols, monoterpenes, norisoprenoids and carotenoids) (Bernardo *et al.*, 2018). The most common abiotic stresses comprise drought (water deficit), salinity, soil acidification, high temperatures and excessive radiation exposure, although it is difficult to discriminate the individual impacts of each stress in an open-field situation.

Moreover, Figures 2 and 3 show the resulting dendrogram associated with heat map. Hierarchical cluster analysis, based on the volatiles versus the non-

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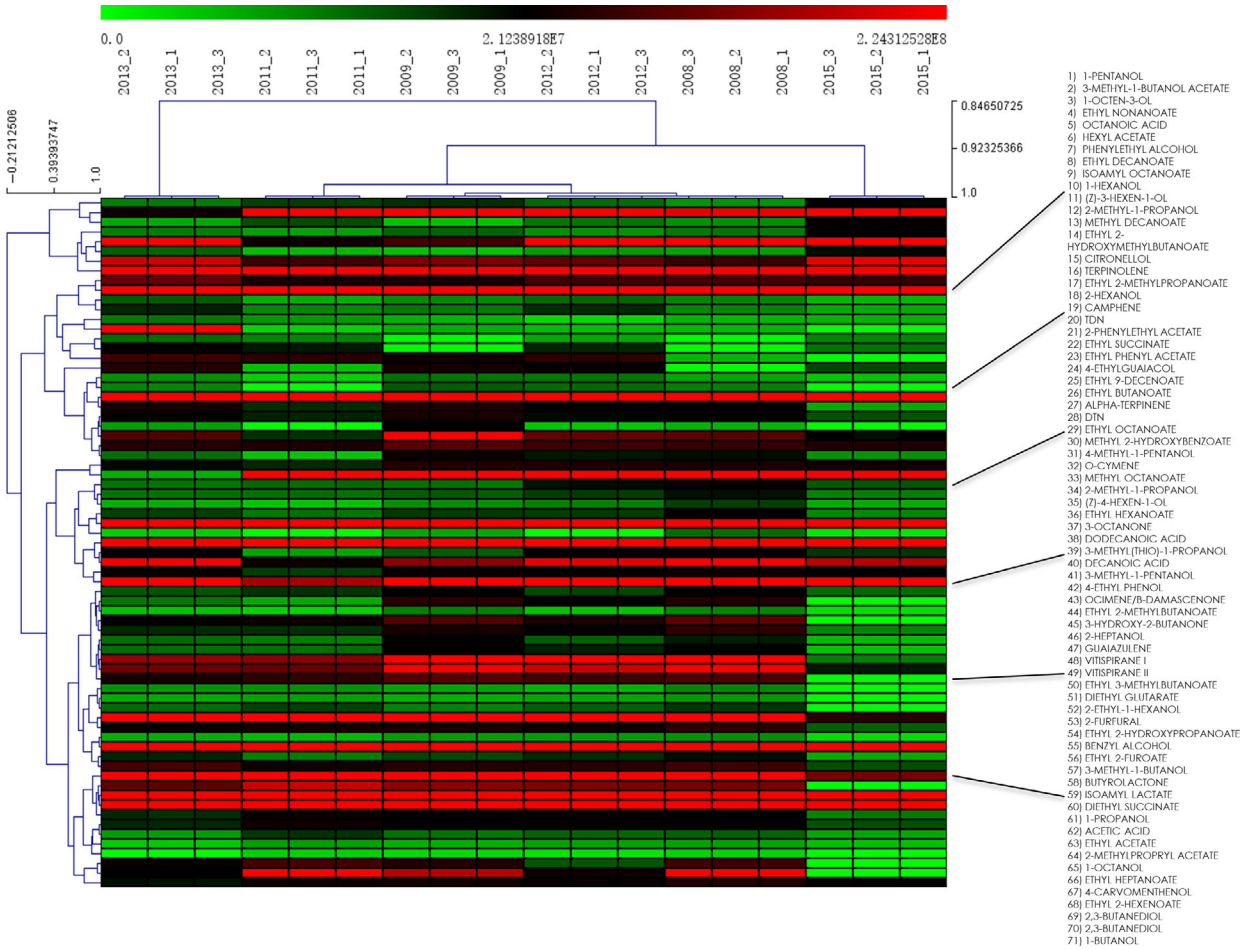

Figure 2 Hierarchical cluster analysis (HCA). Heat map generated by using volatile compounds data. [Colour figure can be viewed at wileyonlinelibrary.com]

volatiles, highlighted a different clustering in relation to the vintages. Considering volatiles, vintages were grouped in four clusters. The first cluster includes 2008, 2009 and 2012 vintages, highlighting major similarities within the same wine samples; the second cluster contains 2011 vintage; the third and fourth clusters 2015 and 2013 vintages, respectively. Conversely, considering non-volatiles, vintages were grouped in four different clusters compared with the previous ones. 2008 and 2012 vintages fall into the first cluster followed by the second one containing 2013 vintage; the third cluster contains 2013 vintage; 2009 and 2015 vintages fall into the fourth cluster. Wine produced in 2009 showed a greater similarity with those of the 2008 and 2012 vintages if we take into consideration the volatile component while, considering the non-volatile compounds, similarity was lower. A similar behaviour occurred for the 2013 vintage, showing high similarity with non-volatiles and low with volatiles.

Regarding volatiles, the compounds dendrograms showed three clusters characterised by a high similarity: (i) ethyl phenyl acetate, 4-ethylguaiacol, ethyl 9-decenoate; (ii) 2-heptanol, guaiazulene, vitispirane I, vitispirane II; and (iii) ethyl 2-furoate, 3-methyl-1-butanol (Figure 2).

Two significant clusters, on the other hand, were highlighted with regard to the non-volatiles: (i) five anthocyanins; and (ii) caffeic acid, p-coumaric acid and gallocatechin gallate (Figure 3).

Conclusions

Volatiles and non-volatiles composition of Vespolina wines has been significantly affected by environmental factors, mainly climatic conditions. The differences recorded in the six vintages both in the temperatures and rainfall clearly influenced grapes composition and consequently the characteristics of the wines. The data

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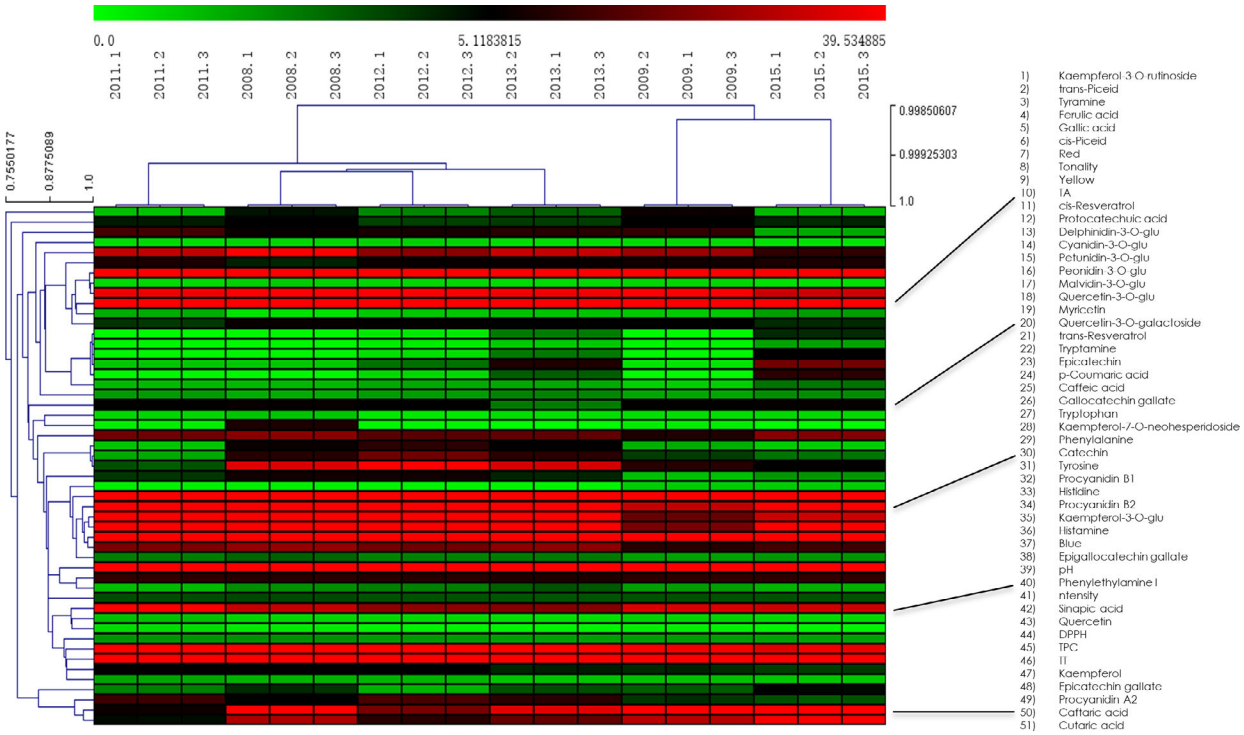

Figure 3 Hierarchical cluster analysis (HCA). Heat map generated by using polyphenols data. [Colour figure can be viewed at wileyonlinelibrary.com]

obtained showed that the qualitative volatile profile from different vintages was similar, but their relative abundance showed slight differences. 2-Phenylethyl acetate, ethyl nonanoate, 2-hexanol, isoamyl octanoate and ethyl 2-hydroxymethylbutanoate resulted the primary compounds responsible for Vespolina wines classification, mainly indicative of wines produced in the 2015 and 2013 vintages. High values of methyl octanoate, 3-methyl-1-butanol, o-cymene, 3-methyl-1-pentanol and ethyl 3-methylbutanoate were characteristic of wines produced in the 2008 and 2009 vintages. Vespolina wines from 2013 and 2015 vintages showed the highest content of anthocyanins, the latter also of caftaric and coutaric acid. Conversely, wines from 2008 showed highest values of procyanidin B1, catechin and gallic acid. *Trans*-piceid, *trans*-resveratrol, caffeic acid, gallocatechin gallate and tryptamine resulted the main compounds responsible for classification.

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Author contribution

Matteo Bordiga: Conceptualization (lead); Formal analysis (equal); Writing-original draft (equal). **Rosa Perestrelo:** Formal analysis (equal); Writing-original draft (equal). **José S. Câmara:** Supervision (equal). **Qiong-Qiong Yang:** Data curation (equal). **Harold Corke:** Supervision (equal). **Fabiano Travaglia:** Formal analysis (equal). **Monica Locatelli:** Supervision (supporting). **Marco Arlorio:** Supervision (supporting). **Jean Daniel Coisson:** Supervision (supporting).

Conflict of interest

There are no conflicts of interest to declare.

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Ethical guidelines

Ethics approval was not required for this research.

Peer review

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Data availability statement

Research data are not shared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Total GC peak area of chemical families identified in Vespolina wines from different vintages.

Figure S2. Distribution of chemical families identified in Vespolina wines from different vintages.

Figure S3. PCA of the volatile compounds (left) and polyphenols, biogenic amines and amino acids (right) of Vespolina wines according to vintage ($n = 3$ for each data point).

Table S1. Volatile compounds identified by HS-SPME/GC-qMS in Vespolina wines from different vintages.

Table S2. Physical-chemical characterization of Vespolina wines.

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