ORIGINAL RESEARCH ARTICLE

The remarkable effects of the non-volatile matrix of wine on the release of volatile compounds evaluated by analysing their release to the headspaces

Ricardo Lopez, Yan Wen, Vicente Ferreira

Laboratory for Aroma Analysis and Enology, Instituto Agroalimentario de Aragón (IA2), Department of Analytical Chemistry, Faculty of Sciences, Universidad Zaragoza, E-50009 Spain

▸ This article is published in cooperation with IVAS 2022 (In Vino Analytica Scientia conference), 3-7 July 2022, Neustadt, Germany.

correspondence:
riclopez@unizar.es

ABSTRACT

Six Spanish wines with different wine-making styles were completely dearomatised and later reconstituted to their original volume with a standard volatile solution containing 15 wine aroma compounds with broad differences in physicochemical characteristics. The headspace composition of the reconstituted wines was evaluated using an automated dynamic headspace (DHS) method combined with thermal desorption (TD) and gas chromatography–mass spectrometry (GC–MS). This method provides a snapshot of the volatile profiles transferred to the headspace in non-equilibrium conditions. The results showed that the non-volatile matrix of the wine significantly affected the transference to the headspace of the 15 aroma compounds. Differences between wines for butanoic and hexanoic acids, DMS and vanillin are above factors 5, 4 or 3, respectively, while for ethyl acetate, ethyl decanoate, 3-methylbutan-1-ol, 2-phenylethan-1-ol or 4-ethylphenol are close to a factor 2. Only ethyl butanoate was uniformly transferred. The release of DMS was related to copper levels, while pH explained part of the release of fatty acids. However, most effects of volatility are difficult to explain. Results strongly indicate that a sample-specific correction for volatility is required to interpret the sensory effects of aroma volatiles.

KEYWORDS: headspace, aroma release, flavour–matrix interactions, wine, GC–MS

INTRODUCTION

Wine aroma is one of the most important characteristics of wine that determines consumer appreciation. Therefore, much of the literature since the early 1970s has been devoted to the identification and quantification of volatile components to understand their contribution to wine aroma. However, more recent research has focused on the fact that many odorants may be involved in complex physicochemical interactions with wine matrix components (Pozo-Bayón & Reineccius, 2009). Such volatile matrix interactions would determine the proportion of aroma compounds in the liquid phase that are effectively released into the headspace. This means that the same aroma composition in two different wines could potentially produce a misinterpretation of the sensory impact owing to large differences in their specific volatilities and concentrations in the headspace.

Numerous studies have investigated the influence of wine matrix components and volatile compounds. The suppressive effect of polyphenolic compounds on wine odorant release has been explained in terms of π–π interactions and hydrophobicity, but as reviewed recently (Pittari *et al.*, 2021), they are not sufficient to explain all observed phenomena, for example, salting-out effects (Mitropoulou *et al.*, 2011). Molecular interactions of polysaccharides with wine odorants have also been studied, with particular attention to the influence of mannoproteins (Jones-Moore *et al.*, 2022). Retention effects related to the protein portion of the polysaccharide combined with the hydrophobicity of the aroma compound seem to be the driving factors that influence volatility (Lubbers *et al.*, 1994). Recently, it has also been shown that amino acids can interact with wine volatiles to influence the flavour of red wine (Espinase Nandorfy *et al.*, 2022). Other matrix compounds can act as "aroma-binders" exerting strong retention effects. This is the case for sulfur dioxide, which can form adducts with carbonyl aroma compounds, such as aldehydes (Daniel *et al.*, 2004; de Azevedo *et al.*, 2007), or cations such as Cu^{2+} that can complex volatile sulfur compounds (Franco-Luesma & Ferreira, 2014). Finally, the ethanol content can affect the behaviour of aroma compounds by decreasing their volatility (Conner *et al.*, 1998) or by other physicochemical effects (Aznar *et al.*, 2004).

Most of the aforementioned studies have been carried out using model solutions that allow the estimation of the influence of individual compounds or specific families on odorant release. However, wine is a complex matrix and this simplified approach, although very useful, does not cover higher-order interactions between wine components and aroma compounds. Therefore, several studies have addressed

TABLE 1. Compounds in the standard aroma mixture, log P and concentration in the reconstituted wines.

Compound	Log P	Boiling point (°C)	Concentration (mg/L)	Aroma vector
Dimethyl sulfide	0.92	37	0.05	Spice woody
Ethyl acetate	0.73	77	27.5	Alcoholic solvent
Ethyl butanoate	1.85	121	0.85	Fruity
Ethyl hexanoate	2.83	168	0.79	Fruity
Ethyl decanoate	4.79	245	1.00	Fruity
Linalool	2.97	198	0.73	Flowery
3-methylbutan-1-ol	1.16	131	238	Alcoholic solvent
2-phenylethan-1-ol	1.36	225	50.3	Alcoholic solvent
3-hydroxybutan-2-one	−0.36	146	0.99	Lactic acid
β-Damascenone	4.21	275	0.53	Fruity
Butanoic acid	0.79	164	0.91	Lactic acid
Hexanoic acid	1.92	205	3.69	Lactic acid
(E)-whisky lactone	2.00	238	0.84	Spice woody
4-Ethylphenol	2.58	218	0.89	Animal leather
Vanillin	1.21	285	0.89	Spice woody

this problem by studying the influence of real wines with different compositional profiles on volatiles. One well-known study is that of Sáenz-Navajas *et al.* (2010), who swapped the volatile and non-volatile fractions of six different wines. Surprisingly, they demonstrated that a red wine matrix was able to make a white wine aroma fraction smell similar to red wine and vice versa. Rodríguez-Bencomo *et al.* (2011) worked with a deodorised non-volatile matrix of five different wines to which volatile compounds were added at different concentrations. Compared with a control model wine without non-volatile components, they observed retention effects for some compounds that were more pronounced in a reconstituted sparkling wine. They also found a salting-out effect for more polar or highly volatile compounds in aged red and sweet wines. Zapata *et al.* (2012) found significant differences in the relative volatilities of 15 out of 20 odorants in 20 wines of different styles. Wen *et al.* (2018) studied the evolution of the headspace composition above 4 different wines observing that release trends for some compounds were significantly related to the wine.

Despite all the published literature, the understanding of the influence of the matrix on the sensory perception of wine flavour is still far from complete. Therefore, this study aimed to measure the influence of the non-volatile matrix of real wine styles on the release of typical wine odorants. The strategy to evaluate this influence would be the use of a previously developed DHS–TD–GC–MS method (Wen *et al.*, 2018) capable of providing a snapshot of the contents in wine vapours. However, instead of studying the original wines, the current study was carried out on reconstitutions made of deodorised non-volatile matrices and a common standard liquid odorant composition.

MATERIALS AND METHODS

1. Reagents and chemicals

Ethanol and dichloromethane were supplied by Merck (Darmstadt, Germany), while tartaric acid 99 % was obtained from Panreac (Barcelona, Spain). The internal standards (methyl 2-methylbutanoate, 2,6-dichloroanisole) were obtained from Sigma-Aldrich (Madrid, Spain). The standard compounds listed in Table 1 with purity above 98 % in all cases were purchased from Sigma-Aldrich (Steinheim, Germany), Fluka (Buchs, Switzerland) and Panreac (Barcelona, Spain). These aroma compounds were mixed together and added to the wine reconstitutions at typical concentration ranges detected in Spanish commercial wines (San Juan *et al.*, 2012) (Table 1).

2. Wine samples

A set of six Spanish wines with different wine-making styles and marked sensory differences were selected, all but one were commercially available. The wines were a 2-year-old Macabeo white wine fermented in stainless steel vats (YW); a 2-year-old Chardonnay white wine fermented and aged in an oak barrel under its lees (CB); a one-year-old Garnacha red wine with a light body from D.O. Cariñena (YR); a five-year-old Tempranillo Reserva red wine from

D.O. Ribera del Duero (24 months in oak barrel) (PE); a six-year-old Tempranillo Reserva red wine from D.O.C. La Rioja (12 months in oak barrel) (CU). A non-commercial pressed red wine was selected due to its intense astringency (PR). Moreover, a synthetic wine (SY) was prepared with 12 % vol of ethanol, and 5 g/L of tartaric acid, adjusted to pH 3.4 with 1 M NaOH.

3. Sample preparation

The non-volatile matrices were prepared as described in the previous works of our laboratory with slight modifications (De-La-Fuente-Blanco *et al.*, 2016; Sáenz-Navajas *et al.*, 2010). Briefly, a 250 mL sample of each wine was poured into a 500 mL rounded flask to remove ethanol and major volatile compounds by a rotary evaporator at 24 °C for 30 min. The distilled sample was frozen at –20 °C and then was further lyophilised by a LyoQuest 85 freeze dryer system (Telstar, Tarrasa, Spain). The resulting syrup was re-dissolved in 20 mL of hydro-alcoholic solution (12 % vol of ethanol). Finally, three successive liquid–liquid extractions by dichloromethane were applied to achieve the complete elimination of all odour compounds. After removing the remained solvent by the rotary evaporator, the obtained liquid was re-dissolved in 250 mL of synthetic wine to its initial volume and analysed by the DHS–TD–GC–MS method mentioned in section 5 to estimate the volatile elimination efficiency and the absence of dichloromethane. After that, each of the deodorised wine matrices was spiked with the corresponding amount of aroma compounds (Table 1) to reconstitute six wine models that contained the same concentration of aroma compounds but different non-volatile matrices. A synthetic wine model was also prepared in the same way.

4. Wine analysis

The free and total sulfur dioxide of each reconstituted wine was determined by the Rankine method recommended by OIV (International Organization of Vine and Wine) (OIV, 2009). Total polyphenol index (TPI) was estimated as absorbance at 280 nm by a UV-VIS spectrophotometer UV-17000 Pharma Spec (Shimadzu, Japan) (Ribéreau-Gayon *et al.*, 2006). Total acidity was measured by titration with 0.1 M NaOH. The non-volatile matrices were weighted after lyophilisation. Copper, iron, manganese and zinc were analysed by Inductively Coupled Plasma–Mass Spectrometry (Vela *et al.*, 2017).

5. Headspace analysis

The headspace composition of each of the six reconstituted wines and the synthetic wine was analysed in triplicate by a previously validated DHS–TD–GC–MS method (Wen *et al.*, 2018). Five mL of sample were pipetted into a 20 mL standard headspace vial, then 20 μL of the internal standard solution (containing methyl 2-methylbutyrate and 2,6-dichloroanisole) were added to reach a concentration level of 200 μg/L. The vial was then closed and placed in the Gerstel MPS2 auto-sampler (Mülheiman der Ruhr, Denmark) where the DHS sampling was automatically carried out under the conditions detailed in Table 2. Samples were

TABLE 2. Experimental parameters of the DHS system.

Parameters			
Incubation time (min)	5	Initial TDU temperature (°C)	20
Incubation temperature (°C)	30	End TDU temperature (°C)	300
Purge volume (mL)	100	Rate TDU (°C/min)	200
Purge flow (mL/min)	25	Initial CIS temperature (°C)	–100
Purge temperature (°C)	40	End CIS temperature (°C)	250
Dry volume (mL)	50	Rate CIS 1 (°C/s)	16
Dry flow (mL/min)	10	Rate CIS 2 (°C/s)	12
Dry temperature (°C)	40	Sample volume (mL)	5
Sorbent material	Tenax TA	No stirring	

subjected to an incubation time of 5 minutes at 30 °C before headspace analysis. Thermal desorption and cryo-focusing were carried out using a Thermo Desorption Unit (TDU) and Cooling Injection System (CIS4) also supplied by Gerstel. Solvent venting mode was used to perform the desorption. Detailed experimental conditions are shown in Table 2.

Gas chromatography–mass spectrometry analysis was performed with a 7890 Agilent GC system coupled with a 5975C Agilent quadrupole mass spectrometer (Santa Clara, CA, USA). A J&W DB-Wax column was used (60 m × 0.25 mm i.d. × 0.25 m film thickness, Agilent). The temperature program was: initial oven temperature at 35 °C held for 3 min, then raised to 220 °C at 10 °C/min, and 7 min of final hold time. The carrier gas was helium at a constant flow of 1 mL/min. The chromatograms were collected in both full scan and SIM mode. Ionization was carried out in electronic impact mode at 70eV. The ion source temperature was 230 °C. Spectra were recorded both in scan mode from 33 to 250 m/z and in selected ion monitoring. Selected ions for particular compounds are detailed in reference (Wen *et al.*, 2018). The headspace content for each compound was normalised against the response of the internal standards as described in our published work (Wen *et al.*, 2018).

6. Aroma release characterisation

A total of 40 mL for each reconstituted wine sample was transferred into a 250 mL uncovered glass beaker to simulate a standard wine serving. Then, 5 mL wine samples were collected from the beaker and transferred to the headspace vial for DHS analysis as mentioned above. The analyses were carried out in triplicate for each wine.

7. Data analysis

XLSTAT (Addinsoft, Paris, France, version 2019) software was used for the statistical analysis. One-way ANOVA on the volatile's concentration was conducted to assess the influence of the different wine matrices on the headspace composition. Principal component analysis (PCA) was carried out to examine the relationship between wine matrix composition and volatile compounds in the headspace. A heat map visualisation of the dataset was also carried out with the same software. The heat map was calculated using ascendant hierarchical clustering based on Euclidian distances.

RESULTS AND DISCUSSION

In the present study, vapours emanating from a set of seven reconstituted wine samples were assessed by dynamic headspace combined with thermal desorption and gas chromatography-mass spectrometry analysis (DHS–TD–GC–MS). The samples were prepared by mixing a standard volatile solution (Table 1) and non-volatile fractions of wines with different characteristics and were re-dissolved to their original volume in 12 % ethanol. It is important to note that the analytical system provides a snapshot of the vapour phase whose composition does not resemble the one corresponding to the thermodynamic equilibrium in a close system, such as the ones obtained in static headspace systems, in stirred SPME or in dynamic headspace systems with intense stirring (Escudero *et al.*, 2014; San-Juan *et al.*, 2010). On the one hand, the short purging time ensures that the volatile composition in the liquid phase will not be much changed in the process. On the other hand, the lack of stirring and the relatively large volume of vapour sampled (100 mL for a 5 mL liquid sample) ensures that the vapour composition is affected by processes of mass transfer from the inner liquid to the surface and through this one (Wen *et al.*, 2018). This situation seems to reflect much better normal orthonasal olfaction in which the least polar and most volatile compounds are transferred to the headspaces at rates well below those

TABLE 3. Conventional oenological parameters of the studied wines.

*TPI: Total polyphenol index

Wine matrix	Abbr.	Years since vintage	Non-volatile residue (g)	Total SO2 (mg/L)	Free SO2 (mg/L)	pH	Total acidity (tartaric acid g/L)	TPI*	Cu (μg/L)	Fe (μg/L)	Zn (μg/L)	Mn (μg/L)
Synthetic wine	SW	–	–	–	–	3.40	5.00	–	–	–	–	–
Young red	YR	1	9.15	30.4	22.9	3.55	4.80	56.5	542	1576	226	521
Young white	YW	2	6.45	129.6	31.1	3.10	5.97	10.3	517	1325	732	452
Under lees white	CB	2	8.40	99.4	38.6	3.46	4.88	14.3	37	843	933	1096
Press wine	PR	1	9.87	17.6	8.6	3.67	4.99	77.2	34	551	670	909
12-month-barrel aged red	CU	6	9.90	40.0	22.2	3.47	4.58	51.9	36	1111	274	457
24-month-barrel aged red	PE	5	10.7	25.6	6.8	3.72	4.91	64.0	98	703	252	603

observed in equilibrium conditions, while the relatively least volatile more polar compounds are less affected (Escudero *et al*., 2014). Because the aroma composition in the liquid phase was identical for all reconstituted wines, such snapshots would provide quantitative information about how the different matrices affect the release of aroma compounds and hence, orthonasal aroma. The compounds in the standard volatile solution (Table 1) were selected from common wine aroma compounds due to their marked differences in chemical and physical properties, including polarity, acid–base characteristics and boiling points. Additionally, they represent typical aroma compounds from different aroma vectors, as recently defined (Ferreira *et al*., 2022)..

The standard volatile solution was added to the non-volatile dearomatised matrices isolated from several wines. These wines were selected to reflect different production styles and sensory characteristics. Two of them were 2-year-old white wines, one was a Macabeo monovarietal fermented in stainless steel vats (YW), and the other was a Chardonnay monovarietal fermented and aged in oak barrels under its lees (CB). The rest of the samples were red wines: a light body young red wine elaborated with Garnacha grapes (YR), a young highly astringent non-commercial pressed red wine (PR), a 6-year-old (12 months in oak barrel) medium body and little astringency Tempranillo wine (CU) and a 5-year-old (24 months in oak barrel) full-body Tempranillo wine (PE). Finally, a synthetic wine (SY) was added as a control. A summary of the oenological parameters of the studied wines is provided in Table 3. YW yielded the least amount of non-volatile residue as expected for a dry white wine. The other white wine (CB) produced a higher quantity of residue, likely because of the presence of mannoproteins

extracted during ageing on lees. There were no large differences in residue among the red wines. TPI was the highest in the pressed wine, followed by the full-body aged wine (PE). As expected, white wines had the lowest values of this parameter. YW was the wine with the highest total acidity and the lowest pH. Finally, some differences were observed in metal content, iron and copper concentrations were markedly higher in young red and white wines. The copper level was particularly high in YW and YR. Although the ethanol concentration was different in the original wines, during this experiment it was maintained at the same level for all the reconstituted wines to avoid the well-known influence of this component (Conner *et al*., 1998).

The headspace volatile responses for each compound and matrix are listed in Table 4. The results of the one-way ANOVA used to assess the influence of the wine matrix are also included in Table 4. According to these results, the effect of the matrix factor was significant ($p \leq 0.0005$ or less) for all compounds, indicating that although the liquid concentrations were initially identical, the release and concentration of the volatile compounds in the headspace were clearly different. Furthermore, the relative variations in headspace concentrations differed depending on the volatile compound studied.

To facilitate the discussion, the data in Table 4 are also presented in the form of bar plots in Figures 1–3. As can be seen in Table 4 and Figure 1, dimethyl sulfide was released to the headspace with marked differences between wines and following a completely different pattern from that observed in the rest of the volatile compounds. The two young wines, both containing maxima amounts of copper (Table 3),

TABLE 4. Volatile response in the headspace at t = 0 min in each reconstituted wine matrix. Values are expressed as average relative areas (n = 3) together with the standard deviation of the mean (s/√3). Codes are SW: synthetic wine; YR: young red; YW: young white; CB: under lees white; PR: press wine; CU: 12-month-barrel aged red; PE: 24-month-barrel aged red. Different letters within each row denote significant differences between wines at p < 0.05 by Fisher's test after a significant one-way ANOVA.

*Significant differences. ANOVA (p ≤ 0.0005); **Significant differences. ANOVA (p ≤ 0.0001)

| Compound	SY	YR	YW	CB	PR	CU	PE
Dimethyl sulfide**	0.00130 ± 0.00003b	0.00054 ± 0.00001c	0.00065 ± 0.00001c	0.00125 ± 0.00005b	0.00199 ± 0.00012a	0.00200 ± 0.00008a	0.00193 ± 0.00208a
Ethyl acetate**	9.69 ± 0.39a	6.35 ± 0.20cd	8.59 ± 0.53ab	9.38 ± 0.57ab	10.24 ± 0.18a	7.80 ± 0.18bc	4.83 ± 5.03d
Ethyl butanoate*	0.151 ± 0.001abc	0.141 ± 0.001bc	0.140 ± 0.002c	0.152 ± 0.004ab	0.161 ± 0.003a	0.144 ± 0.003bc	0.138 ± 0.144bc
Ethyl hexanoate**	2.35 ± 0.02a	2.37 ± 0.02a	2.02 ± 0.05c	1.96 ± 0.05cd	2.24 ± 0.04b	1.90 ± 0.02d	1.78 ± 1.75e
Ethyl decanoate**	4.11 ± 0.05a	2.01 ± 0.12c	2.18 ± 0.57c	2.33 ± 0.18c	3.08 ± 0.03b	1.76 ± 0.08c	1.75 ± 1.61c
3-hydroxybutan-2-one**	0.00096 ± 0.00006d	0.00183 ± 0.00001a	0.00095 ± 0.00003d	0.00117 ± 0.00002bcd	0.00110 ± 0.00004cd	0.00142 ± 0.00012b	0.00130 ± 0.00133bc
β-damascenone**	0.281 ± 0.002a	0.293 ± 0.001a	0.256 ± 0.009b	0.261 ± 0.005b	0.238 ± 0.002c	0.216 ± 0.003d	0.219 ± 0.225d
(E)-whisky lactone**	0.0108 ± 0.0001c	0.0140 ± 0.0002a	0.0105 ± 0.0002c	0.0114 ± 0.0001b	0.0100 ± 0.0001d	0.0094 ± 0.0002e	0.0106 ± 0.0108c
Linalool**	0.185 ± 0.001b	0.235 ± 0.003a	0.167 ± 0.002d	0.173 ± 0.004c	0.162 ± 0.002de	0.157 ± 0.002e	0.160 ± 0.167d
Butanoic acid**	0.00191 ± 0.00002d	0.00591 ± 0.00002b	0.00706 ± 0.00019a	0.00638 ± 0.00028b	0.00114 ± 0.00014e	0.00116 ± 0.00031e	0.00343 ± 0.00263c
Hexanoic acid**	0.0100 ± 0.0002d	0.0193 ± 0.0003c	0.0284 ± 0.0024a	0.0248 ± 0.0010b	0.0067 ± 0.0005e	0.0055 ± 0.0009e	0.0095 ± 0.0080de
3-methylbutan-1-ol**	26.3 ± 0.1b	18.6 ± 0.1d	19.6 ± 0.4d	23.4 ± 0.2c	24.2 ± 0.2c	28.7 ± 0.8a	15.9 ± 15.4e
2-phenylethan-1-ol**	0.162 ± 0.000de	0.286 ± 0.004a	0.210 ± 0.003b	0.166 ± 0.001d	0.153 ± 0.003e	0.167 ± 0.007d	0.194 ± 0.191c
4-ethylphenol**	0.0625 ± 0.0006b	0.0900 ± 0.0014a	0.0626 ± 0.0017b	0.0649 ± 0.0007b	0.0561 ± 0.0005c	0.0557 ± 0.0007c	0.0563 ± 0.0587c
Vanillin**	0.000109 ± 0.000005cd	0.000245 ± 0.000006a	0.000134 ± 0.000004b	0.000104 ± 0.000006de	0.000088 ± 0.000010e	0.000085 ± 0.000008e	0.000133 ± 0.000132bc
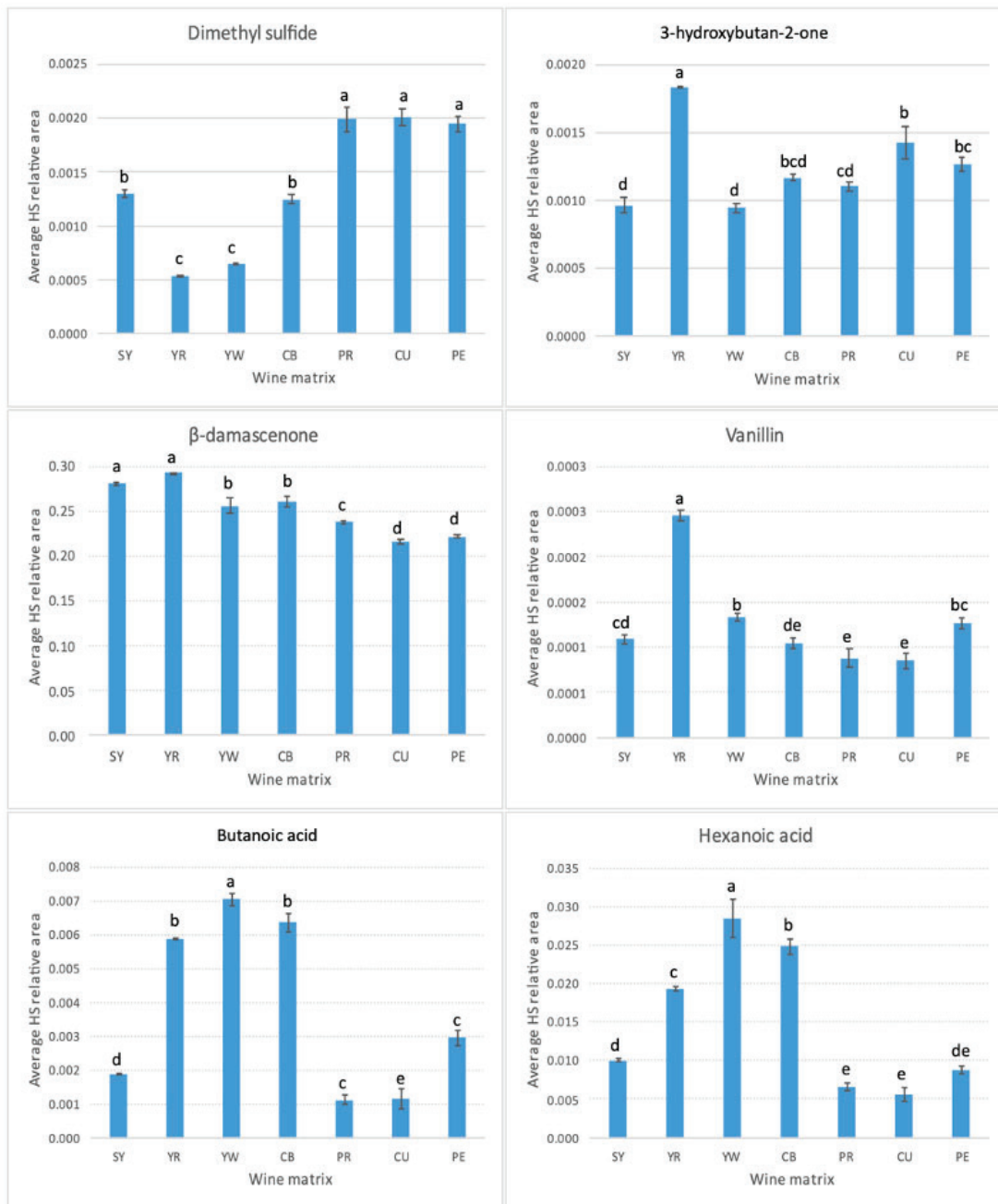
FIGURE 1. Relative areas in the headspace above the reconstituted wines for dimethyl sulfide, 3-hydroxybutan-2-one, β-damascenone, vanillin, and butanoic and hexanoic acids. The graphs show the means of three replicates per wine. The error bars represent the standard error of the means. Different letters indicate significant differences between wines at $p < 0.05$.

released comparatively less than half of the amount released by the synthetic wine and between 3–4 times less than those released by the other red wines. Only the white wine aged on lees released this compound at rates similar to the synthetic wine. Given the remarkable properties of dimethyl sulfide as an aroma enhancer (Escudero *et al*., 2007) and blackcurrant aroma contributor (Lytra *et al*., 2014), the 4-times reduction observed between the maximum and minimum headspace concentrations of dimethyl sulfide could be sufficient to modify the sensory perception of wine. To the best of our

knowledge, it is the first time that this strong influence has been reported. It should be noted that it has been previously reported that DMS levels in the headspace are affected by the presence of copper (Franco-Luesma & Ferreira, 2014). However, in such a study, carried out by static headspace analysis, levels of DMS in the headspace of a synthetic wine decreased just 13–14 % in the presence of Cu(II), while those of H_2S and other mercaptans were completely depleted. Therefore, in that study, it was concluded that Cu-complexation would have just a minor influence on the
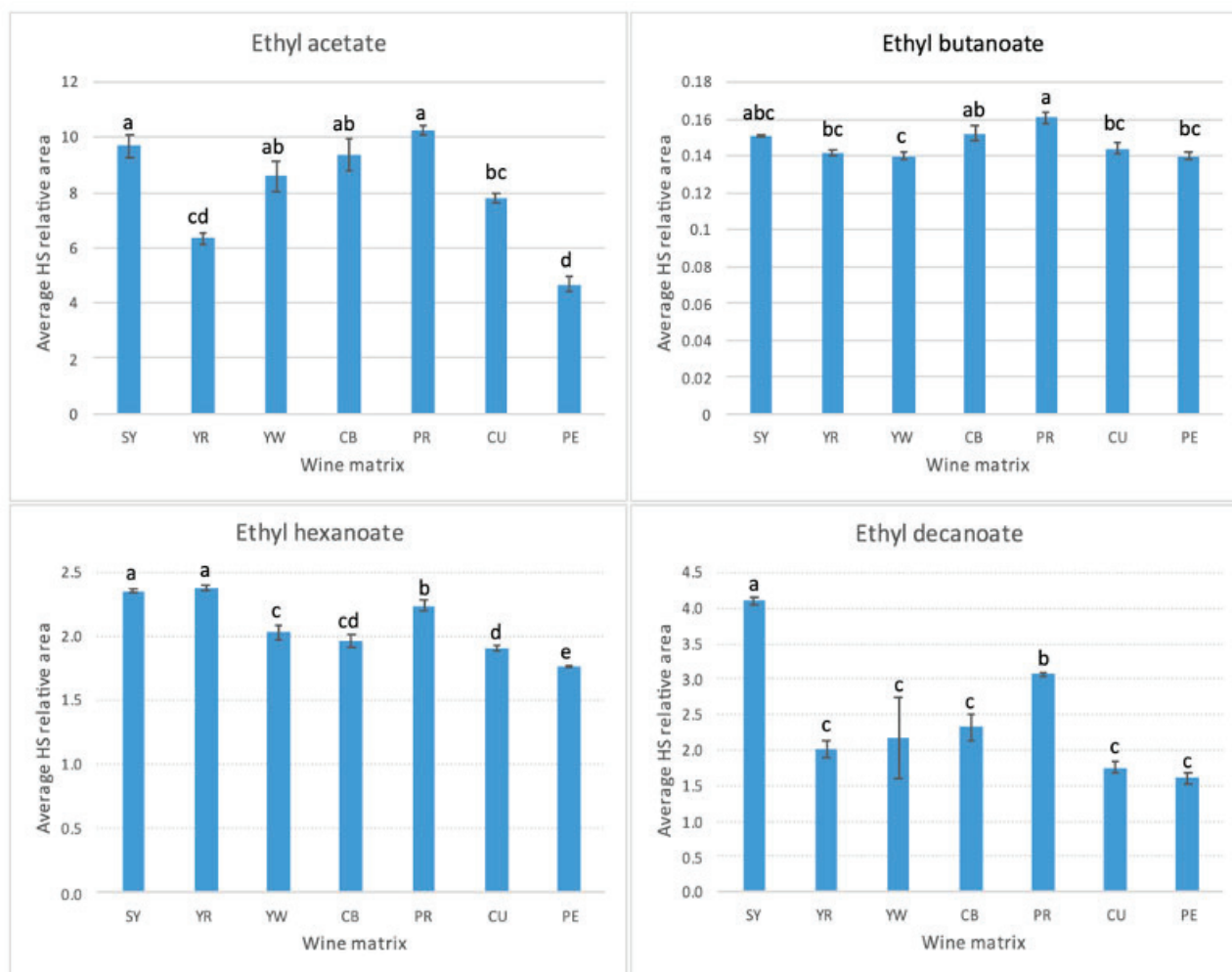
FIGURE 2. Relative areas in the headspace above the reconstituted wines for the ethyl esters. The graphs show the means of three replicates per wine. The error bars represent the standard error of the means. The different letters indicate significant differences between wines at p < 0.05.

perception of DMS. However, the results presented here, demonstrate that it could be of paramount importance. The significantly higher release observed in the three red wines containing low or moderate amounts of copper, in comparison with the control, suggests a decrease in the solubility of this compound caused by the polyphenolic material.

The four ethyl esters added with the standard aroma solution were not homogenous in their release from the wine matrices, as could be expected due to their marked differences in hydrophobicity (Table 1). Furthermore, it has been reported before that volatility of esters has great variability among wines, especially for heavier esters (Zapata *et al.*, 2012). Ethyl decanoate, the most hydrophobic, least soluble and most volatile in the wine matrix of the set (log P = 4.79), showed a clear retention effect in the reconstituted wines. Compared with the synthetic wine model, its headspace concentration was reduced by more than 50 % (Figure 2). This type of decreased release has previously been observed on ethyl decanoate when adding skin tannin extract to a model wine solution (Mitropoulou *et al.*, 2011), and coincides with the retention effect previously observed in some dearomatised

wines (Rodríguez-Bencomo *et al.*, 2011) and with the wine-decay trends reported by (Wen *et al.*, 2018). It should be remarked, however, that such an effect could have a double cause: a) lipophilic retention or, b) poor mass transfer. It has been previously reported that hydroalcoholic solutions (synthetic wines) can transfer volatiles to the headspace far more efficiently than wines due to convectional effects caused by perturbations of the ethanol monolayer at the air–liquid interface upon quick ethanol evaporation in simple solutions (Marangoni effect) (Tsachaki *et al.*, 2009). The fact that differences between wines are just marginal suggests that these mass transfer effects may be dominant. Only the press wine (PR) showed a significantly higher release of ethyl decanoate than the other wines (nearly twice that observed for PE).

Ethyl butanoate and ethyl hexanoate showed statistically significant but quantitatively very small variations in release among the different reconstituted wines. Release levels were particularly homogeneous for ethyl butanoate. Levels transferred ranged from 0.14 to just 0.16, which is consistent with previous observations about the volatilities
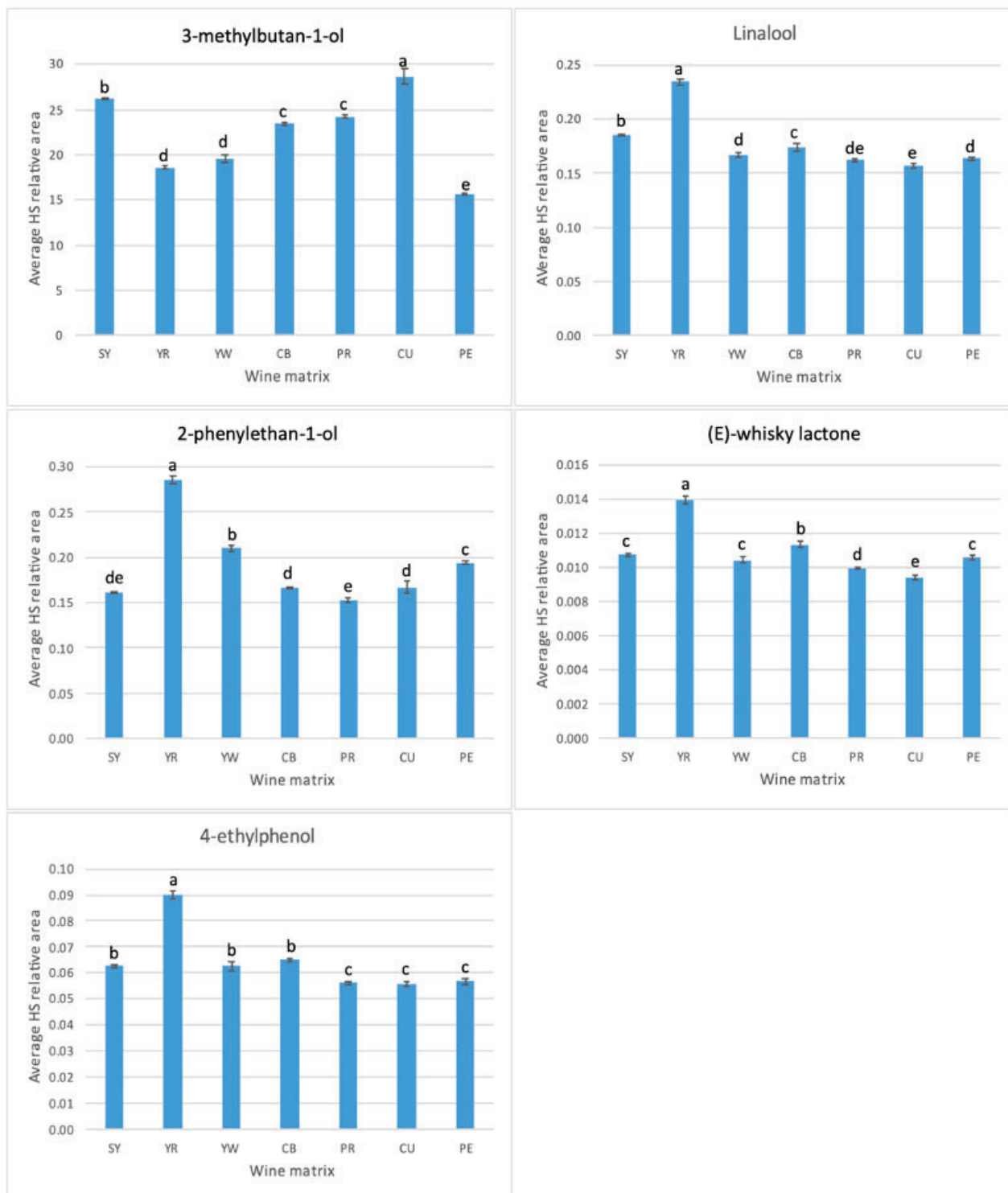
FIGURE 3. Relative areas in the headspace above the reconstituted wines for 3-methylbutan-1-ol, linalool, 2-phenylethan-1-ol, (E)-whisky lactone and 4-ethylphenol. The graphs show the means of three replicates per wine. Error bars represent the standard error of the means. Different letters indicate significant differences between wines at $p < 0.05$.

of this type of compound. In fact, methyl 2-ethylbutanoate was chosen as an internal standard for headspace analysis since its volatility was found to be constant and independent of the type of wine (Bueno *et al*., 2014). In the case of ethyl butanoate, slight retention effects caused by catechin (Lorrain *et al*., 2013) or by a red wine matrix have been reported (Sáenz-Navajas *et al*., 2010), but a salting-out effect caused by an aged red wine matrix has also been reported (Rodríguez-Bencomo *et al*., 2011), which would confirm that, on average, its transference to the headspace is fairly constant. This contrasts with the strong reduction in volatility noted in tannin solutions (Cameleyre *et al*., 2021), which

reveals the difficulty in generalising results obtained in model solutions to real wines. A moderate retention effect of ethyl hexanoate in white wine and sparkling wine has been previously observed (Rodríguez-Bencomo *et al*., 2011).

Interestingly, ethyl acetate, the more polar ester of the group (log P = 0.73), showed marked differences between the different samples. The synthetic wine and the press wine PR released amounts twice the ones released by PE and YR (Figure 2). The differences between the four esters suggest the involvement of several mechanisms determining the release to the headspace. It has been reported that depending on the polyphenol content there is a change in the behaviour of ester release around log P = 2.85 (Pittari *et al*., 2021). In addition, other macromolecules, such as polysaccharides can affect volatile release according to polarity. Assuming a higher mannoprotein content in CB wine (aged on lees), it can be seen that the higher the hydrophobicity of the ester the higher the retention effect on this wine as proposed by (Lubbers *et al*., 1994).

Two ketones (3-hydroxybutan-2-one and β-damascenone) and one aldehyde (vanillin) were included in this experiment. Carbonyl compounds are highly reactive molecules that are known to interact with many molecules present in wines such as amino acids, proteins, and sulfur dioxide among others (de Azevedo *et al*., 2007). Therefore, we expected to observe differences in the headspace concentrations across the matrices. This was the case for 3-hydroxybutan-2-one, for which the two aged reds (CU and PE) and particularly the young red YR, showed an increased release compared to the synthetic wine. The release was specifically intense in the YR wine, which showed a 2-fold increase in release (Figure 1). A potential explanation for this observation could be the presence of non-volatile adducts of 3-hydroxybutan-2-one with sulfur dioxide that could already be present in the original wines; however, no 3-hydroxybutan-2-one was detected in the matrices after dearomatisation and before the addition of the standard aroma mixture. Consequently, the observed increase can be attributed to the salting-out effect caused by the matrix. Notably, 3-hydroxybutan-2-one is the more polar volatile and the only compound for which minimal transference was observed in the model wine. Similar to 3-hydroxybutan-2-one, vanillin also showed a strong salting-out effect in the young red wine (Figure 1). A similar effect has been reported for young red and sparkling wine matrices (Rodríguez-Bencomo *et al*., 2011). β-damascenone showed a different trend of release compared with the other carbonyls. In this case, all wines, except YR, exhibited a decreased transference when compared with the synthetic wine. Such decreased transference, attributed to the retention of β-damascenone by wine matrices with high tannin concentrations (Rodríguez-Bencomo *et al*., 2011; Villamor *et al*., 2013) or with nucleophiles (Daniel *et al*., 2004), has been described previously.

Butanoic and hexanoic acids were the two compounds for which differences in release were more dramatic. They showed a similar trend across the reconstituted wines (Figure 1) with white (YW, CB) and young red (YR) wines presenting a strong salting-out effect, while the 12-month-barrel aged red and pressed wines showed a retention effect. It is important to consider that neither the magnitude of the observed variations nor their direction could be explained only by differences in the pH. While the wine with the highest release was also the one with the lowest pH, YR and CB had considerably higher pHs and just slightly smaller releases, and PE, with the highest pH was not the one showing the smallest transference. Little is known about the influence of the matrix on the release of acids from wine; however, similar salting-out effects in white wines have been observed for butanoic, hexanoic and octanoic acids (Rodríguez-Bencomo *et al*., 2011; Sáenz-Navajas *et al*., 2010). Based on the data gathered here, the matrix could have a significant sensory impact on the perception of cheesy or soapy notes caused by these organic acids and, indirectly, on the type of fruity note perceived (Ferreira *et al*., 2022).

Two alcohols and one terpenol were included in the standard aroma mixture. Their results can be seen in Figure 3. 3-methylbutan-1-ol and 2-phenylethan-1-ol exhibited completely different release behaviours. In the case of 3-methylbutan-1-ol, all wines except the 12-month-barrel aged red (CU), released amounts below those of the synthetic wine. On the contrary, only three wines (CB, PR and CU) released amounts of the volatile similar to those of the synthetic sample. Since both components have similarly low log P values (Table 1), differences have to be attributed to the secondary interactions specifically displayed by the aromatic ring of 2-phenylethan-1-ol. This would facilitate interaction with polyphenols (Jung *et al*., 2000), but this is completely inconsistent with the higher release observed for this compound in wines. The influence of the components of the wine matrix on the release of alcohol is not clear in the literature. In general, a retention effect has been observed for 3-methylbutan-1-ol and 2-phenylethan-1-ol with increased tannin content (Villamor *et al*., 2013), although salting-out by polyphenols or polysaccharides has also been reported (Mitropoulou *et al*., 2011). Linalool, on the other hand, showed a moderate retention effect compared to the synthetic wine (SY), which is similar to those observed by other authors (Mitropoulou *et al*., 2011; Rodríguez-Bencomo *et al*., 2011; Wang *et al*., 2021). Surprisingly, YR exhibited a strong salting-out effect that coincided with those observed for 2-phenylethan-1-ol, 3-hydroxybutan-2-one and vanillin (Figure 1) and also with those of (E)-whisky lactone and 4-ethylphenol (Figure 3). In fact, (E)-whisky lactone and 4-ethyphenol, which are traditionally associated with the aroma of barrel-aged wine, behaved much alike linalool, which suggests that these three compounds exert relatively similar interactions toward matrix components. A similar trend was observed by GC–O for (E)-whisky lactone (Sáenz-Navajas *et al*., 2010), although other authors have reported a decrease in volatility (Rodríguez-Bencomo *et al*., 2011). The reduced release of volatile phenols caused by polyphenols on volatile phenols has been described previously (Rodríguez-Bencomo *et al*., 2011; Wang *et al*., 2022).
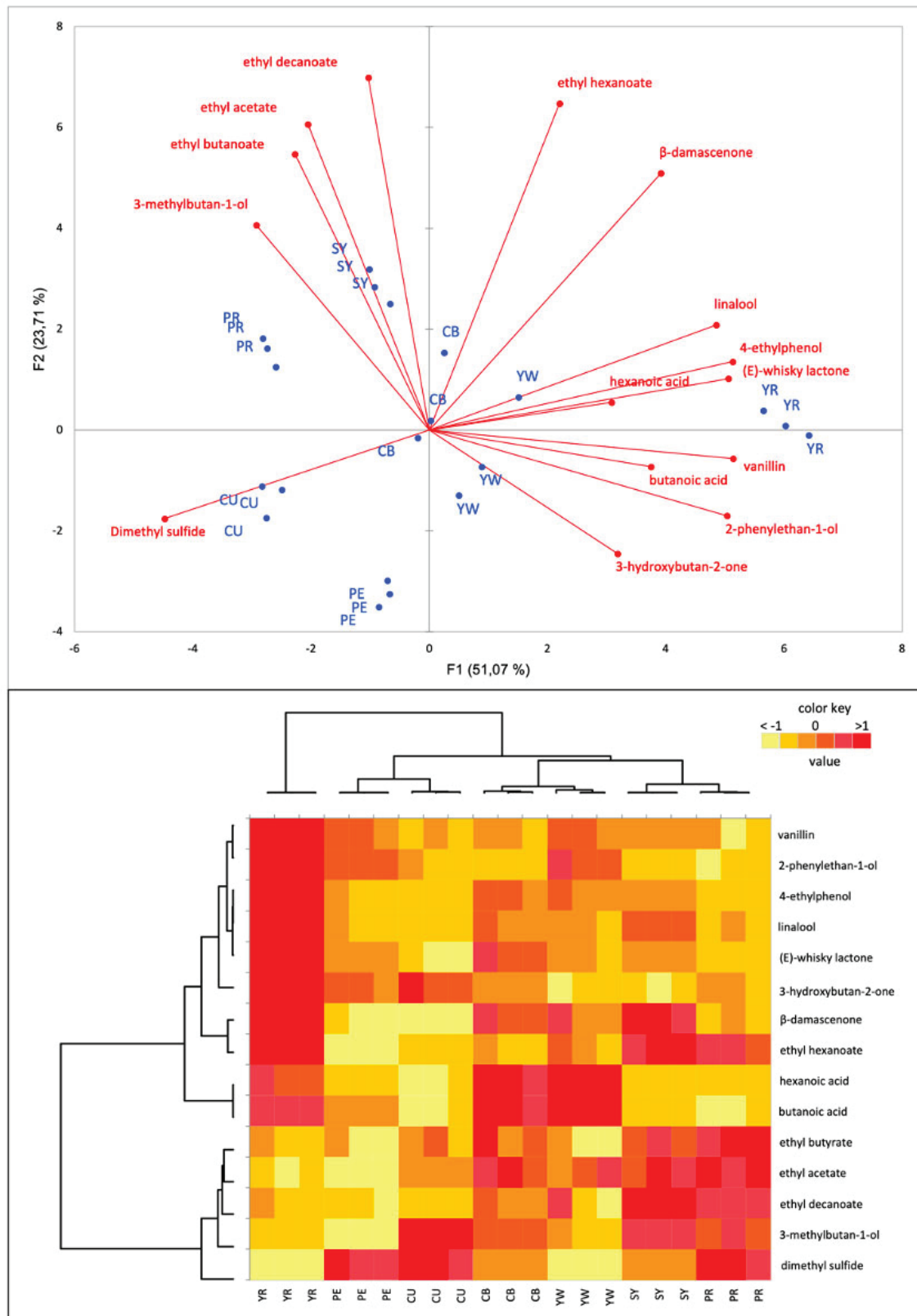
FIGURE 4. Study of relations between wine matrices and released volatile compounds.

(a) Principal Component Analysis BiPlot of volatile profiles of the 7 reconstituted wines. For each wine, the 3 replicates in the score plot are shown with the same label, and the length of the arrows associated with each volatile compound is proportional to its contribution to the overall sample distribution. (b) Heat map matrix summarising the abundance levels of volatile compounds in the headspace of the different reconstituted wines. Within each row, colours are on a scale of light yellow for low abundance to dark red for high abundance. SY: synthetic wine, YR: young red wine, YW: young white wine, CB: under lees white wine, PR: press red wine, CU: 12-month-barrel aged red wine, PE: 24-month-barrel aged red wine.

A principal component analysis (PCA) was carried out to investigate the relationships between the seven matrices (three replicates each) and the 15 added volatiles as a whole (Figure 4a). The PCA was complemented with a heat map visualisation based on the hierarchical clustering of the data (Figure 4b). The PCA biplot of the first two components represented 74.78 % of the total variance. The biplot showed a clear separation between the volatile release profiles of the reconstituted wines; the first dimension (F1, 51.07 %) marked the difference between the young red wine (YR) and the rest of the wines. The young red wine (YR) had the most distinctive pattern of release, being characterised by the highest transference to the headspace of many aroma compounds, including polar (3-hydroxybutan-2-one, vanillin, 2-phenylethan-1-ol) moderately nonpolar ((E)-whisky lactone, 4-ethylphenol, ethyl hexanoate) and strongly non-polar (β-damascenone and linalool), and the smallest transference of DMS. It also had notably high headspace transfer rates for butanoic and hexanoic acid, particularly considering that its pH was 3.55, well above those of the white wines which showed maxima transference for these compounds. The heat map in Figure 4b also shows that 8 out of the 15 volatiles were more abundant in this wine.

Both representations point out that the two white wines (YW and CB) had patterns of release quite similar, leaving aside the smallest transference of DMS observed in YW, most likely due to its huge content in copper (Table 1). White wines (YW and CB) were grouped together in Figure 4b and were characterised by a lower release of dimethyl sulfide and a higher release of acids.

Similarly, barrel-aged red wines (CU and PE) formed a cluster characterised by very poor transferences to the headspace of non-polar compounds, such as ethyl hexanoate, ethyl decanoate, β-damascenone, linalool, (E)-whisky lactone and 4-ethylphenol, the highest release of dimethyl sulfide (together with PR) and low transference of acids. The most notable difference between CU and PE, most surprisingly, is that CU showed the highest transference to the headspace of 3-methylbutan-1-ol and PE the lowest, accounting for the difference almost to a factor of 2. This, without a doubt, could certainly make CU less fruity (Ferreira *et al.*, 2022). The transference of volatiles of the synthetic wine model was characterised by maxima or very high levels of ethyl decanoate, ethyl acetate, ethyl hexanoate and β-damascenone, very low levels of 3-hydroxybutan-2-one and 2-phenylethan-1-ol and intermediate levels of the rest of compounds. The press wine, surprisingly, showed a pattern of volatility comparatively closer to that of the synthetic wine sample and particularly opposite to that of YR, despite both being young red wines. It could be hypothesised that retention effects on PR were less observable due to more intense salting-out effects caused by greater levels of low-weight polyphenols as discussed above.

CONCLUSION

The present work confirms that wine matrix composition exerts a strong effect on the release of volatiles to the headspaces with most likely dramatic sensory consequences. The differences observed between wines for the transference rates to the headspace of volatile fatty acids (butanoic and hexanoic acids), DMS and vanillin are above factors 5, 4 or 3, respectively. Other relevant aroma compounds, such as 3-hydroxybutan-2-one, ethyl acetate, ethyl decanoate, 3-methylbutan-1-ol, 2-phenylethan-1-ol and 4-ethylphenol showed differences around a factor 2 between wines. While some of the effects can easily attributed to a single source, such as a smaller release of DMS by the presence of copper, or a higher release of volatile fatty acids at smaller pHs, matrix effects on volatility seem to be extremely complex and difficult to predict. A light body young red wine released maxima amounts of most volatiles, while a young red press wine followed quite the opposite pattern. Aged red wines tended to release smaller levels of non-polar compounds. These findings strongly suggest that the wine quantitative content of aroma-active compounds, is not enough to adequately interpret sensory properties and that an experimental estimation of volatility in each wine sample should be required.

ACKNOWLEDGEMENTS

REFERENCES

Aznar, M., Tsachaki, M., Linforth, R. S. T., Ferreira, V., & Taylor, A. J. (2004). Headspace analysis of volatile organic compounds from ethanolic systems by direct APCI-MS. *International Journal of Mass Spectrometry*, *239*(1), 17-25. https://doi.org/10.1016/j.ijms.2004.09.001

Bueno, M., Zapata, J., & Ferreira, V. (2014). Simultaneous determination of free and bonded forms of odor-active carbonyls in wine using a headspace solid phase microextraction strategy. *Journal of Chromatography A*, *1369*, 33-42. https://doi.org/10.1016/j.chroma.2014.10.004

Cameleyre, M., Monsant, C., Tempere, S., Lytra, G., & Barbe, J.C. (2021). Toward a Better Understanding of Perceptive Interactions between Volatile and Nonvolatile Compounds: The Case of Proanthocyanidic Tannins and Red Wine Fruity Esters—Methodological, Sensory, and physicochemical Approaches. *Journal of Agricultural and Food Chemistry*. https://doi.org/10.1021/acs.jafc.1c02934

Conner, J. M., Birkmyre, L., Paterson, A., & Piggott, J. R. (1998). Headspace concentrations of ethyl esters at different alcoholic strengths. *Journal of the Science of Food and Agriculture*, *77*(1), 121-126. https://doi.org/10.1002/(SICI)1097-0010(199805)77:1%3C121::AID-JSFA14%3E3.0.CO;2-V

Daniel, M. A., Elsey, G. M., Capone, D. L., Perkins, M. V., & Sefton, M. A. (2004). Fate of damascenone in wine: The role of SO2. *Journal of Agricultural and Food Chemistry*, *52*(26), 8127-8131. https://doi.org/10.1021/jf048582h

De Azevedo, L. C., Reis, M. M., Motta, L. F., da Rocha, G. O., Silva, L. A., & de Andrade, J. B. (2007). Evaluation of the formation and stability of hydroxyalkylsulfonic acids in wines. *Journal of Agricultural and Food Chemistry*, *55*(21), 8670-8680. https://doi.org/10.1021/jf0709653

De-La-Fuente-Blanco, A., Sáenz-Navajas, M. P., & Ferreira, V. (2016). On the effects of higher alcohols on red wine aroma. *Food Chemistry*, *210*, 107-114. https://doi.org/10.1016/j.foodchem.2016.04.021

Escudero, A., Campo, E., Farina, L., Cacho, J., & Ferreira, V. (2007). Analytical characterization of the aroma of five premium red wines. Insights into the role of odor families and the concept of fruitiness of wines. *Journal of Agricultural and Food Chemistry*, *55*(11), 4501-4510. https://doi.org/10.1021/jf0636418

Escudero, A., San-Juan, F., Franco-Luesma, E., Cacho, J., & Ferreira, V. (2014). Is orthonasal olfaction an equilibrium driven process? Design and validation of a dynamic purge and trap system for the study of orthonasal wine aroma. *Flavour and Fragrance Journal*, *29*(5), 296-304. https://doi.org/10.1002/ffj.3204

Espinase Nandorfy, D., Watson, F., Likos, D., Siebert, T., Bindon, K., Kassara, S., Shellie, R., Keast, R., & Francis, I. l. (2022). Influence of amino acids, and their interaction with volatiles and polyphenols, on the sensory properties of red wine. *Australian Journal of Grape and Wine Research*, *n/a*(n/a). https://doi.org/10.1111/ajgw.12564

Ferreira, V., de la Fuente-Blanco, A., & Saenz-Navajas, M. P. (2022). Wine aroma vectors and sensory attributes. In A. G. Reynolds (Ed.), *Managing Wine Quality. Volume 1: Viticulture and Wine Quality* (Second edition ed., Vol. 1, pp. 3-39). Woodhead Publishing. https://doi.org/10.1016/B978-0-08-102067-8.00008-7

Franco-Luesma, E., & Ferreira, V. (2014). Quantitative analysis of free and bonded forms of volatile sulfur compouds in wine. Basic methodologies and evidences showing the existence of reversible cation-complexed forms. *Journal of Chromatography A*, *1359*, 8-15. https://doi.org/10.1016/j.chroma.2014.07.011

Jones-Moore, H. R., Jelley, R. E., Marangon, M., & Fedrizzi, B. (2022). The interactions of wine polysaccharides with aroma compounds, tannins, and proteins, and their importance to winemaking. *Food Hydrocolloids*(123), 107150. https://doi.org/10.1016/j.foodhyd.2021.107150

Jung, D.-M., de Ropp, J. S., & Ebeler, S. E. (2000). Study of Interactions between Food pHenolics and Aromatic Flavors Using One- and Two-Dimensional 1H NMR Spectroscopy. *Journal of Agricultural and Food Chemistry*, *48*(2), 407-412. https://doi.org/10.1021/jf9906883

Lorrain, B., Tempere, S., Iturmendi, N., Moine, V., De Revel, G., & Teissedre, P.-L. (2013). Influence of pHenolic compounds on the sensorial perception and volatility of red wine esters in model solution: an insight at the molecular level. *Food Chemistry*, *140*(1-2), 76-82. https://doi.org/10.5344/ajev.1994.45.1.29

Lubbers, S., Charpentier, C., Feuillat, M., & Voilley, A. (1994). Influence of Yeast Walls on the Behavior of Aroma Compounds in a Model Wine. *American Journal of Enology and Viticulture*, *45*(1), 29-33.

Lytra, G., Tempere, S., Zhang, S. Y., Marchand, S., de Revel, G., & Barbe, J. C. (2014). Olfactory Impact of Dimethyl Sulfide on Red Wine Fruity Esters Aroma Expression in Model Solution. *Journal International Des Sciences De La Vigne Et Du Vin*, *48*(1), 75-85. https://doi.org/10.20870/oeno-one.2014.48.1.1660

Mitropoulou, A., Hatzidimitriou, E., & Paraskevopoulou, A. (2011). Aroma release of a model wine solution as influenced by the presence of non- volatile components. Effect of commercial tannin extracts, polysaccharides and artificial saliva. *Food Research International*, *44*(5), 1561-1570. https://doi.org/10.1016/j.foodres.2011.04.023

OIV. (2009). OIV-MA-AS323-04A Sulfur Dioxide. *Sulfur Dioxide (Resolution Oeno 377/2009), Compendium of International Methods of Analysis*.

Pittari, E., Moio, L., & Piombino, P. (2021). Interactions between polyphenols and volatile compounds in wine: A literature review on physicochemical and sensory insights. *Applied Sciences*, *11*(3), 1157. https://doi.org/10.3390/app11031157

Pozo-Bayón, M. Á., & Reineccius, G. (2009). Interactions Between Wine Matrix Macro-Components and Aroma Compounds. In M. V. Moreno-Arribas & M. C. Polo (Eds.), *Wine Chemistry and Biochemistry* (pp. 417-435). Springer New York. https://doi.org/10.1007/978-0-387-74118-5_19

Ribéreau-Gayon, P., Dubourdieu, D., & Donèche, B. (2006). *Handbook of enology* (2nd ed.). John Wiley.

Rodríguez-Bencomo, J. J., Muñoz-González, C., Andújar-Ortiz, I., Martín-Álvarez, P. J., Moreno-Arribas, M. V., & Pozo-Bayón, M. Á. (2011). Assessment of the effect of the non-volatile wine matrix on the volatility of typical wine aroma compounds by headspace solid phase microextraction/gas chromatography analysis. *Journal of the Science of Food and Agriculture*, *91*(13), 2484-2494. https://doi.org/10.1002/jsfa.4494

Sáenz-Navajas, M.-P., Sáenz-Navajas, M., Campo, E., Campo, E., Culleré, L., Culleré, L., Fernández-Zurbano, P., Valentin, D., & Ferreira, V. (2010). Effects of the nonvolatile matrix on the aroma perception of wine. *Journal of Agricultural and Food Chemistry*, *58*(9), 5574-5585. https://doi.org/10.1021/jf904377p

San Juan, F., Cacho, J., Ferreira, V., & Escudero, A. (2012). Aroma Chemical Composition of Red Wines from Different Price Categories and Its Relationship to Quality. *Journal of Agricultural and Food Chemistry*, *60*(20), 5045-5056. https://doi.org/10.1021/jf2050685

San-Juan, F., Pet'ka, J., Cacho, J., Ferreira, V., & Escudero, A. (2010). Producing headspace extracts for the gas chromatography-olfactometric evaluation of wine aroma. *Food Chemistry*, *123*(1), 188-195. https://doi.org/10.1016/j.foodchem.2010.03.129

Tsachaki, M., Linforth, R. S. T., & Taylor, A. J. (2009). Aroma Release from Wines under Dynamic Conditions. *Journal of Agricultural and Food Chemistry*, *57*(15), 6976-6981. https://doi.org/10.1021/jf901174y

Vela, E., Hernandez-Orte, P., Franco-Luesma, E., & Ferreira, V. (2017). The effects of copper fining on the wine content in sulfur off-odors and on their evolution during accelerated anoxic storage. *Food Chemistry*, *231*, 212-221. https://doi.org/10.1016/j.foodchem.2017.03.125

Villamor, R. R., Evans, M. A., Mattinson, D. S., & Ross, C. F. (2013). Effects of ethanol, tannin and fructose on the headspace concentration and potential sensory significance of odorants in a model wine. *Food Research International*, *50*(1), 38-45. https://doi.org/10.1016/j.foodres.2012.09.037

Wang, S. N., Zhang, Q. T., Zhao, P. T., Ma, Z. Q., Zhang, J. X., Ma, W., & Wang, X. Y. (2022). Investigating the effect of three pHenolic fractions on the volatility of floral, fruity, and aged aromas by HS-SPME-GC-MS and NMR in model wine. *Food Chemistry-X, 13*. https://doi.org/10.1016/j.fochx.2022.100281

Wang, X. J., Li, Y. K., Song, H. C., Tao, Y. S., & Russo, N. (2021). pHenolic matrix effect on aroma formation of terpenes during simulated wine fermentation - Part I: pHenolic acids. *Food Chemistry, 341*. https://doi.org/10.1016/j.foodchem.2020.128288

Wen, Y., Lopez, R., & Ferreira, V. (2018). An automated gas chromatographic-mass spectrometric method for the quantitative analysis of the odor-active molecules present in the vapors emanated from wine. *Journal of Chromatography A, 1534*, 130-138. https://doi.org/10.1016/j.chroma.2017.12.064

Zapata, J., Lopez, R., Herrero, P., & Ferreira, V. (2012). Multiple automated headspace in-tube extraction for the accurate analysis of relevant wine aroma compounds and for the estimation of their relative liquid–gas transfer rates. *Journal of Chromatography A, 1266*, 1-9. https://doi.org/10.1016/j.chroma.2012.10.015.