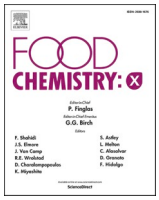
Food Chemistry: X 13 (2022) 100281

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



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

Shengnan Wang [a], Qianting Zhang [a], Pengtao Zhao [a,b,*], Zeqiang Ma [c], Junxiang Zhang [d], Wen Ma [d], Xiaoyu Wang [a,b,*]

[a] College of Food Engineering and Nutritional Science, Shaanxi Normal University, Shaanxi 710119, PR China
[b] Engineering Research Center of High Value Utilization of Western China Fruit Resources, Ministry of Education, Shaanxi Normal University, Shaanxi 710119, PR China
[c] State Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources, College of Animal Science and Technology, Guangxi University, Nanning 530004, PR China
[d] School of Food and Wine, Ningxia University, Yinchuan 750021, PR China

ARTICLE INFO

Keywords:
Wine aroma
Phenolic fractions
Volatility
Matrix effect
Model wine

ABSTRACT

In this study, the volatility of three typical wine aromas in model wine was investigated by HS-SPME-GC-MS, NMR, and sensory evaluation as influenced by different concentrations and structural properties of phenolics. Results showed that three phenolic fractions (phenolic acids, monomeric/oligomeric and polymeric procyanidins) exhibited different matrix effects on floral, fruity, and aged aromas perception. Physico-chemical and sensory analyses together indicated that all fractions reduced the perceived intensity of fruity and aged aroma attributes, and displayed stronger retention effects on fruity aromas at higher mDP and concentrations. Monomeric/oligomeric and polymeric procyanidins promoted highly hydrophobic floral aromas release, whereas inhibiting the volatility of low hydrophobic fruity aromas. NMR confirmed that the reduction in the volatility of rose oxide, ethyl butanoate and whiskey lactone was attributed to interactions with epicatechin. This study aims to provide new thoughts and theoretical support for wine aroma regulation during winemaking by reconstructing the phenolic composition in wine.

Introduction

The perceived wine aroma is playing a role in determining wine organoleptic quality. The distribution and proportion of aroma compounds in wine and its headspace depend mainly on their volatility, which is believed to be strongly affected by the matrix composition including polyphenols, ethanol, polysaccharides, and proteins (Pozo-Bayón & Reineccius, 2009). In particular, the interaction between aroma compounds and polyphenols has been paid more attention to their influences on the odorant volatility, aroma release and overall perception during aging and consumption (Muñoz-González, Martín-Álvarez, Moreno-Arribas, & Pozo-Bayón, 2014). The structural properties and concentration of aroma and phenolic compounds are significant factors influencing the behavior of wine aroma release (Pittari, Moio, & Piombino, 2021). To date, increasing research has been focused on aromas formation and changing during wine consumption, while the effects of polyphenols with varied concentrations and structures on wine aroma release remains unclear.

Floral, fruity, and aged characteristics are typical bouquets that determine the organoleptic quality of most wines. More than 1000 aroma compounds have been identified in wine, ranging in concentrations from ng/L to mg/L (Flanzy, 2003). They are classified as primary, secondary, and tertiary aroma originated from grapes, wine fermentation and the aging process. Aroma compounds including alcohols, esters, aldehydes, ketones, acids, and terpenes are frequently reported as the main contributors of pleasant wine aroma (Tetik, Sevindik, Kelebek, & Selli, 2018). For example, monoterpenes give wine distinctive floral aromas that represent the vinification character of wine grapes, adding complexity to the wine aroma (Jeromel, Korenika, & Tomaz, 2019). Esters like ethyl hexanoate, ethyl octanoate, and ethyl decanoate from yeast metabolic during fermentation present fresh fruity aroma of young wines (Carpena et al., 2020). Moreover, aromatic substances formed during barrel aging, such as guaiacol, whisky lactone, eugenol, and volatile phenols, conferring spicy, toasted, caramel-like notes and typical aged character to the wine (De Rosso, Panighel, Dalla Vedova, Stella, & Flamini, 2009).

* Corresponding authors at: No. 620, West Chang'an Avenue, Chang'an District, Xi'an 710119, PR China.
E-mail addresses: zhaopengtao@snnu.edu.cn (P. Zhao), wangxiaoyu@snnu.edu.cn (X. Wang).

https://doi.org/10.1016/j.fochx.2022.100281
Received 8 January 2022; Received in revised form 2 March 2022; Accepted 5 March 2022
Available online 8 March 2022

Effects of phenolic compositions from wine with different varieties and vintages on aroma release have been particularly focused. Pinot Noir wines with low tannin content are often judged with “red berries” and “flowers” aromatic characteristics, whereas Cabernet Sauvignon wines rich in tannins have characteristic aromas of black currant and green pepper (Longo, Carew, Sawyer, Kemp, & Kerslake, 2021). Young red wines are abundant in monomeric polyphenols that can interact with terpene, thus reducing aroma release under in vitro conditions (Dufour & Bayonove, 1999). The aged wines exhibit different types and degrees of polymerization for tannins, involving different interactions with aroma molecules (Lorrain et al., 2013; Mitropoulou, Hatzidimitriou, & Paraskevopoulou, 2011). Proanthocyanidins in wine were characterized as oligomers or polymers of flavan-3-ols, which were reported as significant contributors to the organoleptic quality of wine. Nevertheless, previous studies on the reaction between polyphenols and aromas used mostly polyphenols from grapes or commercial tannins (Lorrain et al., 2013; Mitropoulou et al., 2011; Villamor, Evans, Mattinson, & Ross, 2013). Information on the influence of polyphenolic fractions extracted from wine, especially those with different structural properties (like mDP) on wine aroma volatility, is still lacking.

It is widely accepted that phenolic compounds act on aroma compounds mainly through weak intermolecular non-covalent interactions like hydrogen bonding, van der Waals forces and hydrophobic effects (Pozo-Bayón & Reineccius, 2009). Some monomeric polyphenols like catechin and epicatechin interact with some aroma compounds via weak hydrophobic and π-π interactions, reducing aroma volatility (Dufour & Bayonove, 1999; Jung & Ebeler, 2003). The strength of the interaction and the presence of π-π stacking, which is stabilized by hydrogen bonds between the galloyl rings of phenolic compounds and the aromatic rings of aroma compounds, are structure-dependent (Aronson & Ebeler, 2004; Jung, de Ropp, & Ebeler, 2000). There are currently many analytical approaches to explain the relationships between these molecules. Headspace solid-phase microextraction (HS-SPME) combined with GC–MS is applied to reveal phenolic compounds’ structure–activity and quantitative effect on aroma compounds from a chemical composition perspective (Cameleyre, Lytra, & Barbe, 2018). Nuclear magnetic resonance (NMR) spectroscopy used to explore the mechanism between phenolic and aroma compounds, as well as to offer information on binding site localization and the nature of interactions at molecular level (Moreau & Guichard, 2006).

In this context, the binding behavior of three phenolic fractions (phenolic acids, monomeric/oligomeric procyanidins, polymeric procyanidins) isolated from wine at different concentrations on three typical wine aromas (floral, fruity, and aged) was investigated in model wine. Physicochemical analysis (HS-SPME-GC–MS, NMR), sensory evaluation, and multivariate analyses (heat-map clustering and partial least squares regression) were used to elucidate the relationship between aroma-phenolic reactions and the perceived strength of aroma attributes. This work aimed to provide more information to understand the effect of polyphenols on the typical aromas and put forward a new clue of improving wine organoleptic quality by regulating phenolic composition through enological strategies.

Materials and methods

Reagents

Aroma standards including phenylethyl alcohol, linalool, rose oxide, β-damascenone, geraniol, nerol, α-terpineol, β-citronellol, ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl cinnamate, isoamyl acetate, ethyl 3-methylbutanoate, eugenol, whiskey lactone, 4-ethylphenol, guaiacol and 3-octanol with purity ≥97% were purchased from Tokyo Chemical Industry (Shanghai, China). Flavan-3-ols standards including (+)-catechin, (−)-epicatechin, (−)-epicatechin-3-O-gallate, (−)-epigallocatechin with purity higher than 98% were supplied by Sigma-Aldrich (St. Louis, MO, USA). Folin-Ciocalteau reagent, *p*-dimethylamino-cinnamaldehyde (DMACA), dimethylsulfoxide (DMSO-d_6 containing 0.03% tetramethylsilane) were supplied from Merck (Darmstadt, Germany). All reagents of analytical grade were provided by Kermel (Tianjin, China), and all solvents of chromatographic grade were from Oceanpak (Gothenburg, Sweden).

Determination of phenolic compounds

Separation of phenolic fractions

Phenolic fractions were separated using Waters C18 Sep-Pak cartridges (Waters, Milford, USA). Sample wines were selected from Cabernet Sauvignon wine (non-oak aging, 2018, Eastern Helan mountains, Ningxia, China) and the elution procedure was as described in our previous experiments (Wang et al., 2021). The fractions F1, F2 and F3 solutions obtained by successive elution with deionized water, ethyl acetate and methanol were phenolic acids, monomeric/oligomeric

Table 1
Key aroma compounds detected in Cabernet Sauvignon wine and their chemical and sensory attributes.

Family of aromas	Compounds	Concentrations (μg/L)	Odor description	[a] Odor thresholds (μg/L)	[b] OAV	[c] Log P (o/w)
Floral	Phenylethyl alcohol	30,000	rose	14,000 (Ferreira, López, & Cacho, 2000)	2.142	1.36
	Linalool	200	citrus, floral, muscat	15 (Guth, 1997)	13	2.97
	Rose oxide	2	floral, rosy	0.2 (Guth, 1997)	10	3.19
	β-Damascenone	5	baked/stewed apple, honey	0.05 (Guth, 1997)	100	4.04
	Geraniol	50	floral	30 (Guth, 1997)	1.67	3.56
	Nerol	50	citrus, floral	300 (Mateo & Jiménez, 2000)	0.17	3.47
	α-Terpineol	50	floral, woody	250 (Ferreira, López, & Cacho, 2000)	0.2	2.67
	β-Citronellol	50	floral	100 (Guth, 1997)	0.5	3.30
Fruity	Ethyl butanoate	1000	pineapple	20 (Guth, 1997)	50	1.80
	Ethyl hexanoate	2000	guava, strawberry, anise	14 (Ferreira, López, & Cacho, 2000)	143	2.85
	Ethyl octanoate	2000	fruity	5 (Ferreira, López, & Cacho, 2000)	400	3.84
	Ethyl decanoate	1000	grape	200 (Ferreira, López, & Cacho, 2000)	5	4.86
	Ethyl cinnamate	20	cinnamon, sweet	1.1 (Ferreira, López, & Cacho, 2000)	18	2.99
	Isoamyl acetate	6000	banana	30 (Guth, 1997)	200	2.25
	Ethyl 3-methylbutanoate	200	fruity	3 (Guth, 1997)	66.7	2.16
Aged	Eugenol	200	clove, honey	14.3 (Ferreira, López, & Cacho, 2000)	14	2.27
	Whiskey lactone	800	coconut	67 (López, Aznar, Cacho, & Ferreira, 2002)	11.94	2.63
	4-Ethylphenol	500	bitumen, leather	440 (López, Aznar, Cacho, & Ferreira, 2002)	1.14	2.58
	Guaiacol	100	smoky, medicine	11 (Ferreira, López, & Cacho, 2000)	9.1	1.32

[a] References for odor thresholds are shown in parenthesis, measured in 10% water/ethanol solution (Guth, 1997) and 11% water/ethanol solution containing 7 g/L glycerol and 5 g/L tartaric acid, pH = 3.4 (Ferreira, López, & Cacho, 2000).
[b] OAV represents odor activity value, which was calculated as the ratio between the concentration of an individual aroma compound in wine to its perception value.
[c] Hydrophobic constants obtained from The Good Scents Company (https://www.thegoodscentscompany.com/).

procyanidins and polymeric procyanidins, respectively (Sun, Leandro, Ricardo, & Spranger, 1998). Finally, the three fractions were vacuum-evaporated to dry and re-dissolved in model wine.

The model wine was prepared with 4 g/L tartaric acid and ethanol (12% v/v) in water, and pH was adjusted to 3.3 with analytical grade sodium hydroxide.

Total phenolic and total flavanol assay

Total phenolic content (TPC) was determined by the Folin-Ciocalteu method and expressed as gallic acid equivalent (mg/L) (Singleton, Orthofer, & Lamuela-Raventós, 1999). Total flavanol content (TFC) was measured by p-DMACA-HCl method and expressed as catechin equivalent (mg/L) (Li, Tanner, & Larkin, 1996).

Structural characteristics of phenolic compound fractions

Structural characteristics of phenolic fractions were determined by HPLC-MS (Agilent 1260-6460, Agilent, CA, USA), which was equipped with a reversed-phase Water XBridge® Shield RP18 column (3.5 μm, 4.6 × 250 mm). The elution program for HPLC was carried out according to the method of Zhang et al. (2020). The mean degree of polymerization (mDP), percentage of galloylation (%G) and percentage of prodelphinidins (%P) were determined following the method by Basalekou et al. (2019) with modification. Briefly, dried phenolic fractions were dissolved in methanol and then was acid-catalyzed by benzyl mercaptan. All samples were replicated three times.

Determination of wine aromas by HS-SPME-GC–MS

Aroma compounds in wine were analyzed by HS-SPME coupled with GC–MS (Agilent 8890-5977B, Agilent Technologies, Inc., CA, USA). One mL of wine, 4 mL of citrate buffer (0.2 mol/L citric acid in saturated sodium chloride solution, pH 2.5), and 20 μL of internal standard (3-octanol at 20 mg/L in methanol) were held in a 20 mL-headspace vial and stirred by a 1 cm Teflon stir bar in a 50℃-water bath for 30 min. Then, the volatiles were extracted by a SPME fiber (50/30 μm DVB/CAR/PDMS, Supelco, Inc., USA) at 50℃ water a bath for 30 min and were desorbed in the gas chromatography injector at 250℃ for 3 min.

Volatile compounds were separated on a DB-wax capillary column (30 m × 0.25 mm × 0.5 μm) and the chromatographic program was carried out using the method described by Zhao, Qian, He, Li, and Qian (2017). The volatile compounds were identified by mass spectra from NIST 17.0 database and retention times compared with pure reference compounds in our lab. All samples were performed in triplicate.

Three types of aroma compounds (floral, fruity, aged aromas) were selected based on GC–MS analysis (Table 1), and combined with references to choose the appropriate concentration of aroma compounds in model wine. Odor activity value (OAV) was calculated by the ratio between the concentration and the odor threshold of aroma compounds found in the bibliography.

Effect of phenolic fractions on the release of aroma compounds in headspace

Phenolic fractions (F1, F2 and F3) were dissolved separately in a model wine to get the final concentration gradient was 67%, 80%, 100%, 133%, 200% and 400% of the original wine concentrations. Aroma compounds with a concentration (refer to Table 1) were then spiked into the solution. The retention and release of three types of aroma compounds in the presence and absence of phenolic compounds were analyzed by HS-SPME-GC–MS, expressed as the relative peak area in relation to that of the same internal standard. The conditions for HS-SPME were slightly modified from 2.3, where the experiments were carried out at room temperature (20℃) with a model wine solution instead of the buffer, and the GC–MS conditions were used the procedure described in 2.3.

Molecular mechanism of phenolic and aroma compounds

To get insight into the molecular mechanism of the reaction between phenolic compounds and three types of aroma compounds, epicatechin standards and aroma compound standards (rose oxide, ethyl butanoate and whiskey lactone) were selected for study by HS-SPME-GC–MS and NMR techniques.

HS-SPME-GC–MS analysis

The retention and release extent of aroma compounds (rose oxide, ethyl butanoate and whiskey lactone) in model wine was determined in the presence and absence of epicatechin. Ten milligrams of epicatechin were added to 5 mL of the aroma solution, and the SPME fiber was exposed to the sample headspace for 10 min at room temperature and desorbed in the GC injector of GC–MS at 250℃ for 3 min. The GC–MS was carried out using the programme described in 2.3.

^{1}H NMR analysis

For ^{1}H NMR analysis, 5 mg/mL of epicatechin solution, aroma compounds (rose oxide, ethyl butanoate and whiskey lactone at concentration of 5 mg/mL) solution and a mixture of epicatechin and aroma compounds (1:1) solution were prepared in DMSO-d_6 and transferred into 5 mm NRM tubes. The ^{1}H NMR spectra were recorded at 400 MHz using a Bruker Avance 500 spectrometer with a 5 mm z-gradient Bruker inverse probe at 25 °C. Tetramethylsilane was used as an internal standard for chemical shift measurements and all spectra were processed using MestReNova 9.1.0 software (Mestrelab, Santiago de Compostela, Spain).

Sensory evolution

Sensory analysis was carried out in model wine and Pinot Noir (oak aging, 2018, Burgundy, France), assessed by a sensory panel of 11 trained members (7 females and 4 males, aged between 20 and 50 years old). Three phenolic fractions extracted from Cabernet Sauvignon according to the method in 2.2.1 were added to the model wine and Pinot Noir at certain concentrations (low, medium, and high) corresponding to 67%, 100% and 200% of the concentration gradient in 2.4. Aroma standards were spiked into the model wine at the concentrations shown in Table 1.

The sensory panel was trained over 70 days to assess the wine aroma using a mixture of aroma standards and the aroma kit ''Le Nez du Vin'' (Jean Lenoir, Provence, France) until the deviation of the tasting group's wine aroma profile analysis was less than 5% of the overall mean. During formal sessions, the panelists were required to orthonasally smell each sample and rate eleven aroma attributes picked in Table 1 (rose, floral, pineapple, sweet, fruity, grape, cinnamon, banana, clove, smoky, coconut) on a 10-point structured scale for each wine (0 = low, 5 = intermediate, 9 = high) (De Castilhos, Del Bianchi, Gómez-Alonso, García-Romero, & Hermosín-Gutiérrez, 2019). Each wine sample (30 mL) was filled at room temperature (20℃) in a standard tasting glass marked with three digits and covered with plastic Petri dishes based on a sequential monadic random arrangement. All wines were evaluated in triplicate.

Statistical analysis

All analysis was performed in triplicates, and the values were expressed as mean values ± standard deviation (SD). Data were statistically analyzed using one-way analysis of variance (ANOVA) with Duncan test at $P < 0.05$, using the software for SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Multivariate analysis was subjected to heat-map cluster analysis and PLS-DA model by Origin Pro 9.0 (Origin Lab Corporation, Northampton, MA, USA) and SIMCA P13software (Umetrics, Umea, Sweden), respectively. The chemical structures of phenolic and aroma compounds were drawn using ChemDraw 15.1 (CambridgeSoft,

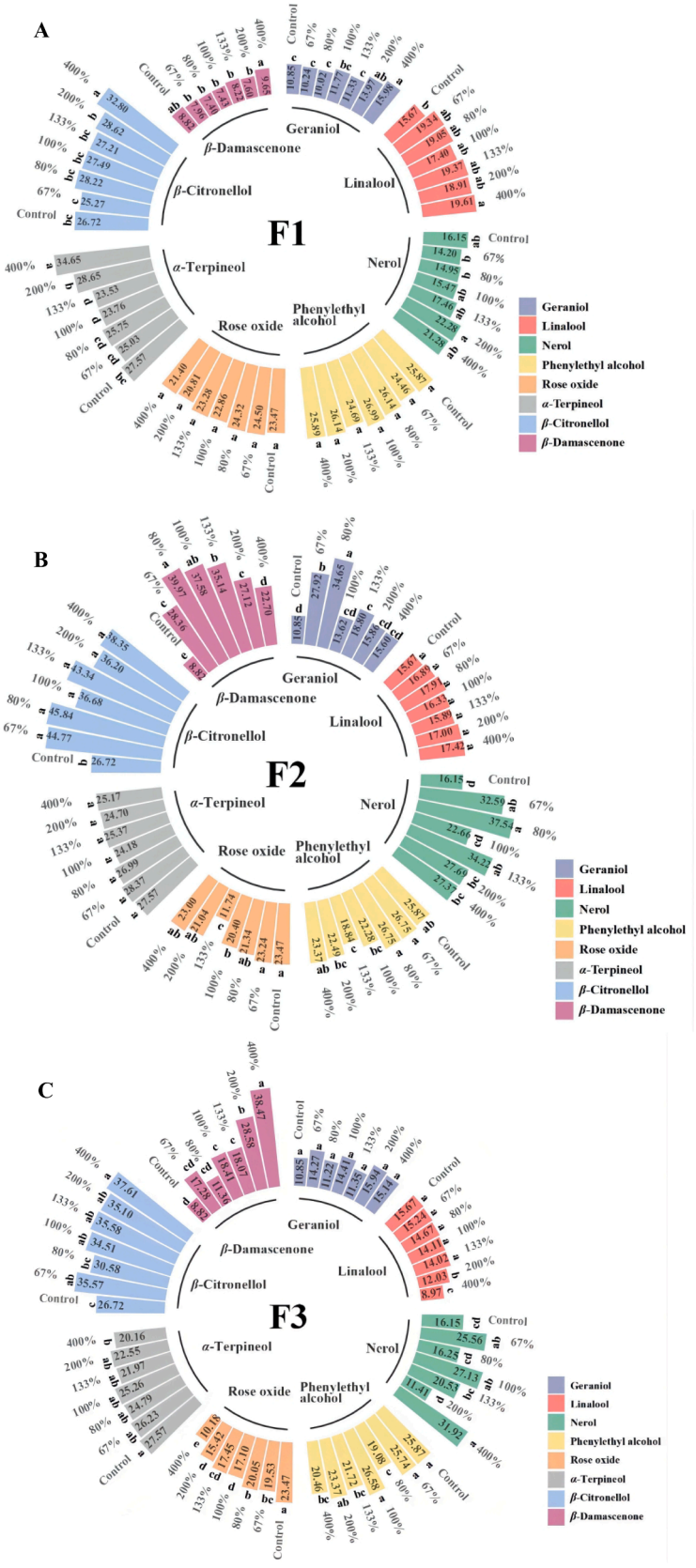


Fig. 1. Effect of phenolic fractions at different concentrations (67%, 80%, 100%, 133%, 200% and 400% of the original wine) on the aroma compounds. Phenolic fractions include F1 (**A**, **D**, **G**), F2 (**B**, **E**, **H**), and F3 (**C**, **F**, **J**). Aroma compounds include floral aroma (**A-C**), fruity aroma (**D-F**), and aged aroma (**G-J**). Control: model wine without phenolic fraction. The height of colored bars (marked on the color bar) represents the ratio calculated by the peak area of target compounds to internal standard in the headspace of model wine (n = 3). Different letters represent significant differences determined using ANOVA, followed by Duncan's test ($p < 0.05$).

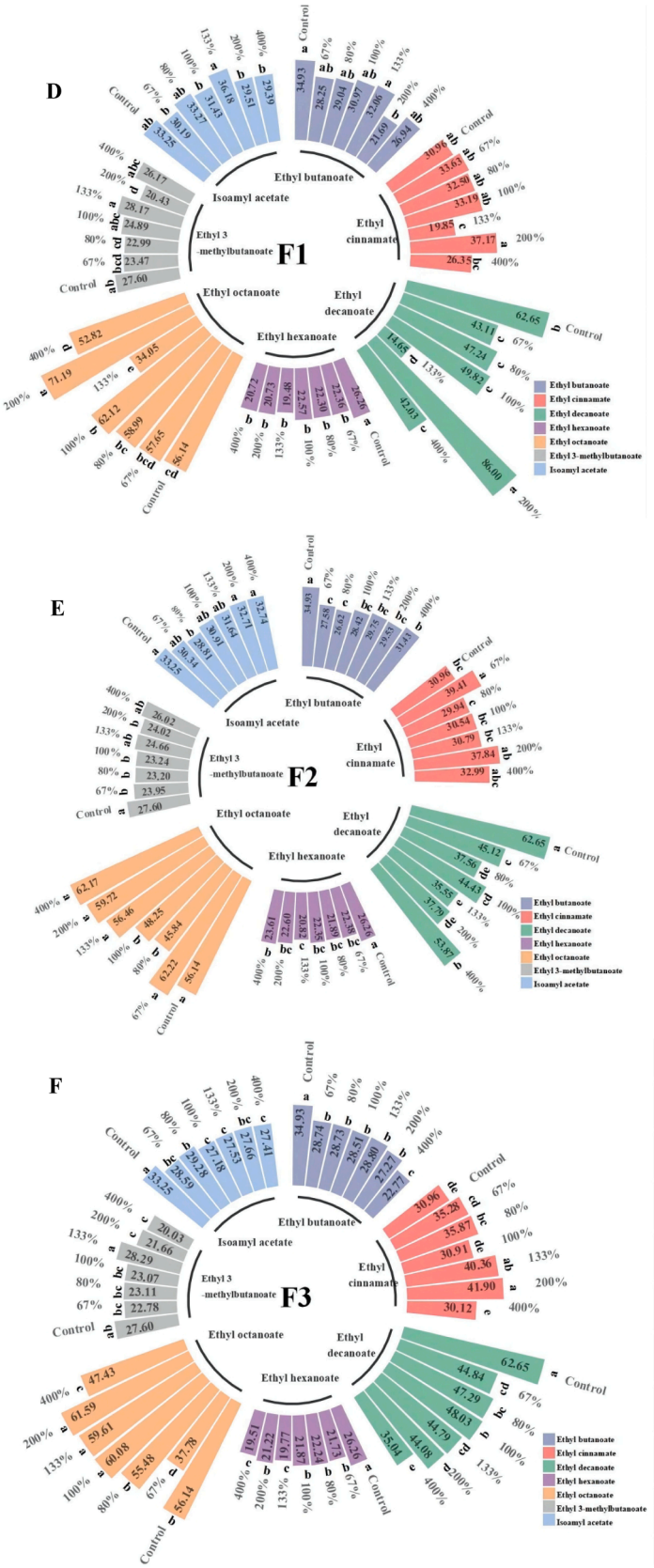


Fig. 1. (*continued*).

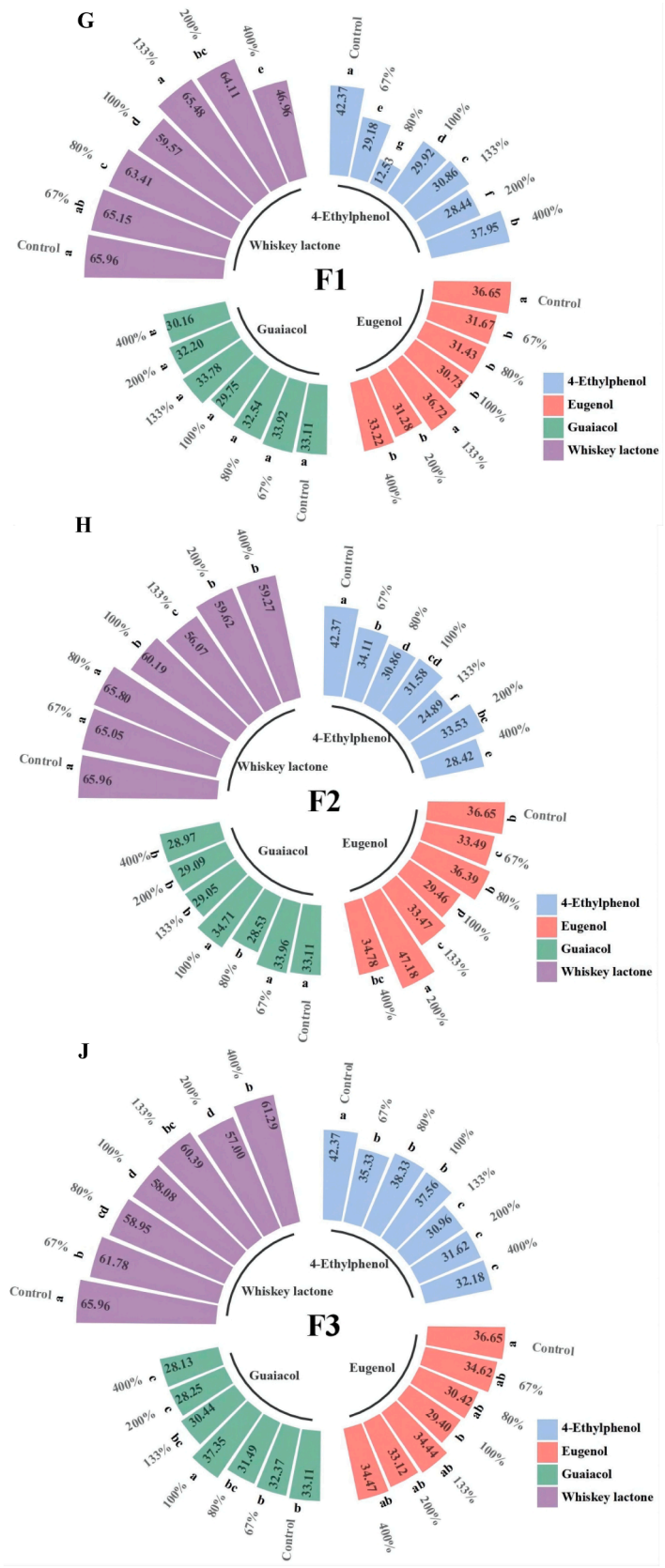


Fig. 1. (*continued*).

Cambridge, MA, USA), and the figures were generated using Origin Pro 9.0 (Origin Lab Corporation, Northampton, MA, USA).

Results and discussion

Characterization of different phenolic fractions

The structural characterization results (see Table S1 in supplementary materials) showed that the TPC of three phenolic fractions (F1, F2, and F3) isolated from the wine accounted for 63.93%, 27.61% and 8.46% of the total, and the TFC of each fraction was 54.63%, 42.56% and 2.81% of the total, respectively. It indicated that polymeric procyanidins were the primary components of the wine. The mDP of F3 (5.44) was detected significantly greater than F2 (2.67). Additionally, subunit composition is also an essential parameter for characterizing the polyphenol structure (Basalekou et al., 2019). Only terminal units of catechin (C), epicatechin (EC), and epigallocatechin (EGC) were found in the F1, especially C and EGC being the main components. The terminal and extended unit of EGC were calculated to be the most predominant subunits in F2, whereas F3 exhibited more extension subunits than terminal subunits, probably related to its higher mDP. After acid-catalyzed depolymerization, F3 released more extension units, mainly EC and EGC. The structural variations among the monomeric phenols are responsible for the different volatility of aroma after reaction with aroma compounds, as reported in previous studies (Dufour & Bayonove, 1999; Jung & Ebeler, 2003). According to the subunit composition, F3 was calculated to contain more prodelphinidins than F2, and the trend in P% values was in accordance with mDP. In contrast, F2 possessed a higher G% value than F3, implying that F2 contained more galloyl groups and that the π-π stacking of phenolic-aroma interactions is stabilized by hydrogen bonding between the galloyl ring of the phenolic compound and the aromatic ring of aroma compounds (Aronson & Ebeler, 2004; Jung et al., 2000).

Effect of phenolic fractions on aroma compounds releasing

Floral aromas

In the case of F1 (Fig. 1 A), the volatility of some aromas (*α*-terpineol and nerol) was reduced at lower concentrations (67% and 80%) of F1 in model wine compared to the control (without polyphenols). Conversely, high concentrations (200% and 400%) of F1 promoted the release of certain aromas like *α*-terpineol, *β*-citronellol, geraniol and linalool, as they were detected at higher amounts in the headspace than the control. This suggested that the volatility of aromas varied according to the concentration of phenolic acid in the wine. The volatility of linalool was measured to be enhanced at all concentrations of F1, whereas the release of the C13 norisoprenoids such as *β*-damascenone was inhibited by F1, except for a slight increase in its release at the highest concentration (400%) of F1. Furthermore, no significant difference between rose oxide, phenylethyl alcohol and control was observed. It indicated that polyphenols might selectively affect the volatility of aromas according to their molecular chemical structure, which is consistent with the findings of Jung and Ebeler (2003).

The volatility of *β*-citronellol, nerol, geraniol and *β*-damascenoneon was increased at all concentrations of F2 compared to the control, indicating a "salting out" effect of F2 on these aromas (Fig. 1 B). At 80% concentration of F2, these aromas were highest levels in the headspace, with their release increasing in the order *β*-damascenone > geraniol > nerol > *β*-citronellol, consistent with the magnitude of their hydrophobic constants (Log P, 4.04 > 3.56 > 3.47 > 3.30). This implied that hydrophobicity was the dominant factor in the interaction of strongly hydrophobic floral aroma with monomerics/oligomerics, which is in line with previous work that the hydrophobicity of polyphenols and aroma compounds was significantly involved in the modification of aroma release (Aronson & Ebeler, 2004). Moreover, a decreasing trend was observed in the effect of F2 on the volatility of rose oxide, which could be the result of the retention of aroma compounds in the wine matrix through interaction with polyphenols.

Fig. 1 C shows that the volatility of rose oxide and *α*-terpineol decreased with increasing F3 concentration, and the aroma release determined in the headspace was lowest at 400% concentration of F3. Higher concentrations of F3 (200% and 400%) inhibited the volatility of linalool and phenylethyl alcohol compared to the control, which is in accordance with previous studies where higher concentrations of tannins generally significantly inhibited the volatility of most aromas (Perez-Jiménez, Chaya, & Pozo-Bayón, 2019; Rodríguez-Bencomo et al., 2011; Villamor et al., 2013). Nevertheless, F3 promoted the release of strong hydrophobic aromas, including *β*-citronellol (Log P = 3.30), *β*-damascenone (Log P = 4.04) and nerol (Log P = 3.47), which is consistent with the findings of F2.

Altogether, the effects of phenolic acids, monomeric/oligomeric, and polymeric procyanidins on these floral aromas were different. All results found that hydrophobicity appeared to be the dominant factor in the reaction of strongly hydrophobic aromas (Log P > 3.30) with polymeric tannin (F2 and F3). Compared to phenolic acids, polymeric tannin promoted the release of the hydrophobic aromas *β*-damascenone and *β*-citronellol at all concentrations, especially the most hydrophobic *β*-damascenone with the highest headspace release. Notably, the presence of phenolic acid slightly enhanced the volatility of linalool, whereas the polymeric procyanidins were measured to inhibit its release. This is probably because the increase in mDP favored the attractive interaction and aggregation of tannins (Riou, Vernhet, Doco, & Moutounet, 2002), resulting in the retention of linalool in the phenolic matrix. Furthermore, some monoterpene aromas may interact with phenolic compounds through weak intermolecular non-covalent interactions, e.g., the presence of polymeric tannin inhibited the volatility of rose oxide.

Fruity aromas

As seen in Fig. 1 D, the less hydrophobic ethyl hexanoate (Log P = 2.85) and ethyl butanoate (Log P = 1.80) exhibited lower levels in the headspace than the control at all concentrations of F1. The presence of F1 also reduced ethyl 3-methylbutanoate (Log P = 2.16) and isoamyl acetate (Log P = 2.25) releases, except for a slight increase in aroma at 133% concentration of F1. It indicated that phenolic acids appeared to have a retention effect on the volatility of the low hydrophobic aromas (Log P < 2.85). The matrix effect of phenolic acids was proven to control the release and modulate the overall character of wine aromas (Wang, Li, Song, Tao, & Russo, 2021). Noteworthy, the more hydrophobic esters ethyl decanoate (Log P = 4.86), ethyl octanoate (Log P = 3.84) and ethyl cinnamate (Log P = 2.99) showed a consistent trend at high concentrations (133%-400%) of F1. The release of these aromas was lowest at an F1 concentration of 133%, highest at a concentration of 200% and further decreased below the control at a concentration of 400%. Of these, the volatility of the most hydrophobic ethyl decanoate was inhibited by the low concentration (67%-100%) of F1 and exhibited a higher retention effect, probably due to the higher interaction with the wine matrix (Rodríguez-Bencomo et al., 2011). On the contrary, the volatility of ethyl octanoate was slightly enhanced at low concentrations (67%–100%) of F1 compared to the control, which is in accordance with previous findings that the volatility of more hydrophobic esters may be more likely to be reduced (Lorrain et al., 2013; Mitropoulou et al., 2011).

The volatility of ethyl hexanoate, ethyl butanoate, ethyl 3-methylbutanoate, isoamyl acetate and ethyl decanoate was decreased in the presence of F2, as shown in Fig. 1 E. In this case, the degree of decrease in the volatility of the low hydrophobic isoamyl acetate (1.61%–4.44%) was lower than the more hydrophobic ethyl decanoate (8.78%–27.10%). Our results suggested that the degree of volatility decrease may be related to the aroma hydrophobicity, which is thought to be the main driving force for the reaction of polyphenols with aroma compounds (Dufour & Bayonove, 1999; Jung & Ebeler, 2003). Furthermore,

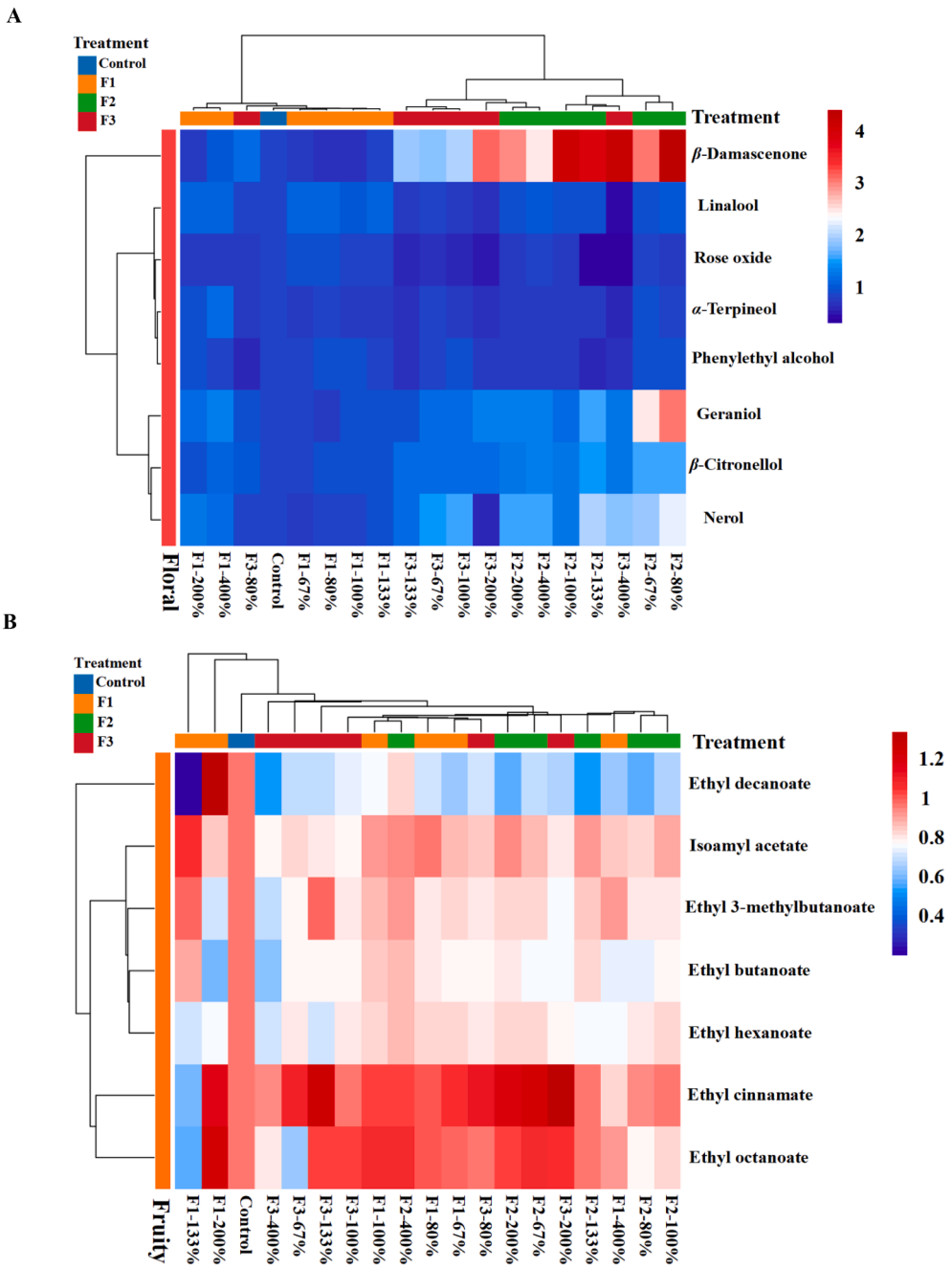


Fig. 2. Heat-map analysis of aroma compounds influenced by different concentrations (67%, 80%, 100%, 133%, 200% and 400% of the original wine) of phenolic fractions. Phenolic fractions: F1-phenolic acids, F2-monomeric/oligomeric procyanidins, F3-polymeric procyanidins. Aroma compounds: floral aroma (**A**), fruity aroma (**B**), aged aroma (**C**). Control treatment group represents model wine without polyphenols. Data were normalized according to the control treatment for each aroma compound, and aroma compound releases were shown on a heat scale that ranges from lower to higher. Clustering of the aroma compound is according to the Centroid's algorithm.

the volatile behavior of ethyl cinnamate was different from that of the other esters, with increased release at F2 concentrations of 67% and 200%. This may be attributed to the chemical structure of aroma compound, with π-π interactions between the aromatic ring and other electronically unsaturated systems in the matrix (Dufour & Bayonove, 1999; Jung et al., 2000).

The volatility of ethyl butanoate, ethyl 3-methylbutanoate, isoamyl acetate, ethyl hexanoate and ethyl decanoate was inhibited by all concentrations of F3, and their headspace levels were lower than the control (Fig. 1 F). This result revealed that all concentrations of polymeric procyanidins could reduce the release of low hydrophobic esters (Log P < 2.85). Among these aroma compounds with decreased volatility, the most hydrophobic ethyl decanoate exhibited a more significant decline in volatility than the less hydrophobic aroma compound, a result that is in agreement with F2. The release of fruity aromas was inhibited by high concentrations (400%) of F3, except for ethyl octanoate, and the release was lowest at this concentration. These results mean that the high mDP phenolic fraction was more likely to retain fruity aroma in the wine matrix, especially at high concentrations. Similar findings were reported for the negative impact of increased levels and the polymerization of polyphenols on the release of esters, corresponding to a significant reduction in the fruity profile of aged red wines rich in polymeric

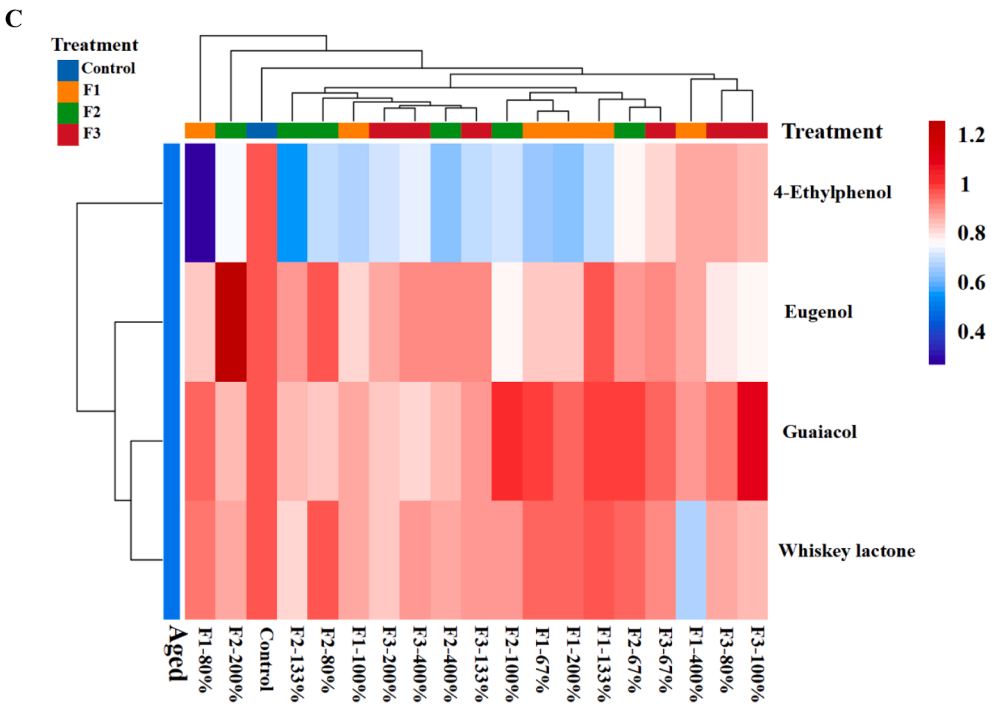


Fig. 2. (*continued*).

tannins (Rodríguez-Bencomo et al., 2011).

In conclusion, the above results indicated that the effect of three phenolic fractions on the fruity aroma tended to be a retention effect, particularly as the monomeric/oligomeric and polymeric procyanidins inhibited the volatility of more ester aromas than phenolic acids. The high mDP proanthocyanidins had the strongest retention effect on fruity aroma at high concentrations. Additionally, it was observed that the interaction between fruity aroma and polyphenols was related to the hydrophobicity of the aroma compounds. With a cut-off point of log P = 2.85, the release of low hydrophobic ester aromas was more likely to be inhibited by three phenolic fractions, whereas the release of high hydrophobic ester aromas increased at certain concentrations.

Aged aromas

The presence of F1 reduced the volatility of 4-ethylphenol and whiskey lactone compared to the control, which had similar hydrophobic constants (Log P values of 2.58 and 2.63, respectively), as shown in Fig. 1 G. The degree of decrease in the volatility of 4-ethylphenol (4.42%–29.84%) was greater than whiskey lactone (0.81%–19.00%), probably due to differences in the chemical structure of aged aroma. Simultaneously, all concentrations of F1 inhibited the volatility of eugenol, except at 133% which showed no significant effect on eugenol. F1 had no significant effect on the volatility of guaiacol.

The volatility of 4-ethylphenol was inhibited by all concentrations of F2, which were significantly lower in the headspace than the control (Fig. 1 H). In contrast, the release of whiskey lactone was only slightly reduced at high concentrations (100%–400%) of F2 compared to the control. The volatility of the less hydrophobic guaiacol displayed a slightly decreasing trend and retention effect at certain concentrations (80% and 133%–400%) of F2. The F2 essentially inhibited the release of eugenol except for an increase in release at 200% polyphenol concentration.

The presence of F3 essentially decreased the volatility of aged aroma, resulting in a lower aroma content in the headspace than the control (Fig. 1 J). The greatest reduction (4.04%–11.41%) in volatility was observed for 4-ethylphenol among these aged aromas, whereas the release of the least hydrophobic guaiacol increased at 100% concentration of F3, consistently with the F2 results. The decrease in volatility of the volatile phenols including 4-ethylphenol, eugenol and guaiacol could be explained by the benzene ring of these aromas interacting with the gallic acyl ring of the polyphenolic component via π-π interactions (Jung et al., 2000).

In summary, the impact of three phenolic fractions on the aged aroma tended essentially to be a retention effect, with the volatility of 4-ethylphenol in particular significantly decreased at all concentrations of F1, F2 and F3, and showing the greatest reduction compared to the other aromas. Previous works also reported a significant linear decrease in the volatility of 4-ethylphenol in model solutions with increasing polyphenol concentrations (Petrozziello et al., 2014).

Discussion of the aroma compounds releasing affected by phenolic fractions

Heat-map cluster analysis

The floral, fruity, and aged aroma compounds releasing into the model wine headspace of phenolic fractions gradients were further classified by heat-map cluster analysis. For floral aroma (Fig. 2 A), the F2 and F3 treatment groups clustered into one group, except for F3 at a concentration of 80%, whereas F1 and the control treatment groups clustered into one group. Of these, the less hydrophobic (Log P < 3.19) linalool, rose oxide, α-terpineol and phenylethyl alcohol clustered into one group, and their F2 and F3 treatment groups were mostly released in lower amounts than the control and F1 treatment groups. Conversely, the F2 and F3 treatment groups of the more hydrophobic (Log P > 3.30) geraniol, β-citronellol, nerol and β-damascenone were mostly released in higher amounts than the control and F1 treatment group, with the highest releases of β-damascenone.

The clustering of fruity aromas verified the results in 3.2, where the interaction of fruity aromas with polyphenols was strongly related to the hydrophobicity of aroma compounds. As seen in Fig. 2 B, ethyl hexanoate, ethyl butanoate, ethyl 3-methylbutanoate and isoamyl acetate clustered first into one group (cluster 1) with their Log P < 2.85, then with ethyl octanoate and ethyl cinnamate (cluster 2, Log P of 3.84 and 2.99, respectively), whereas the most hydrophobic ethyl decanoate (Log P = 4.86) formed its own group (cluster 3). Except for F1 at concentrations of 133% and 200%, the volatility of aromas in cluster 1 mostly

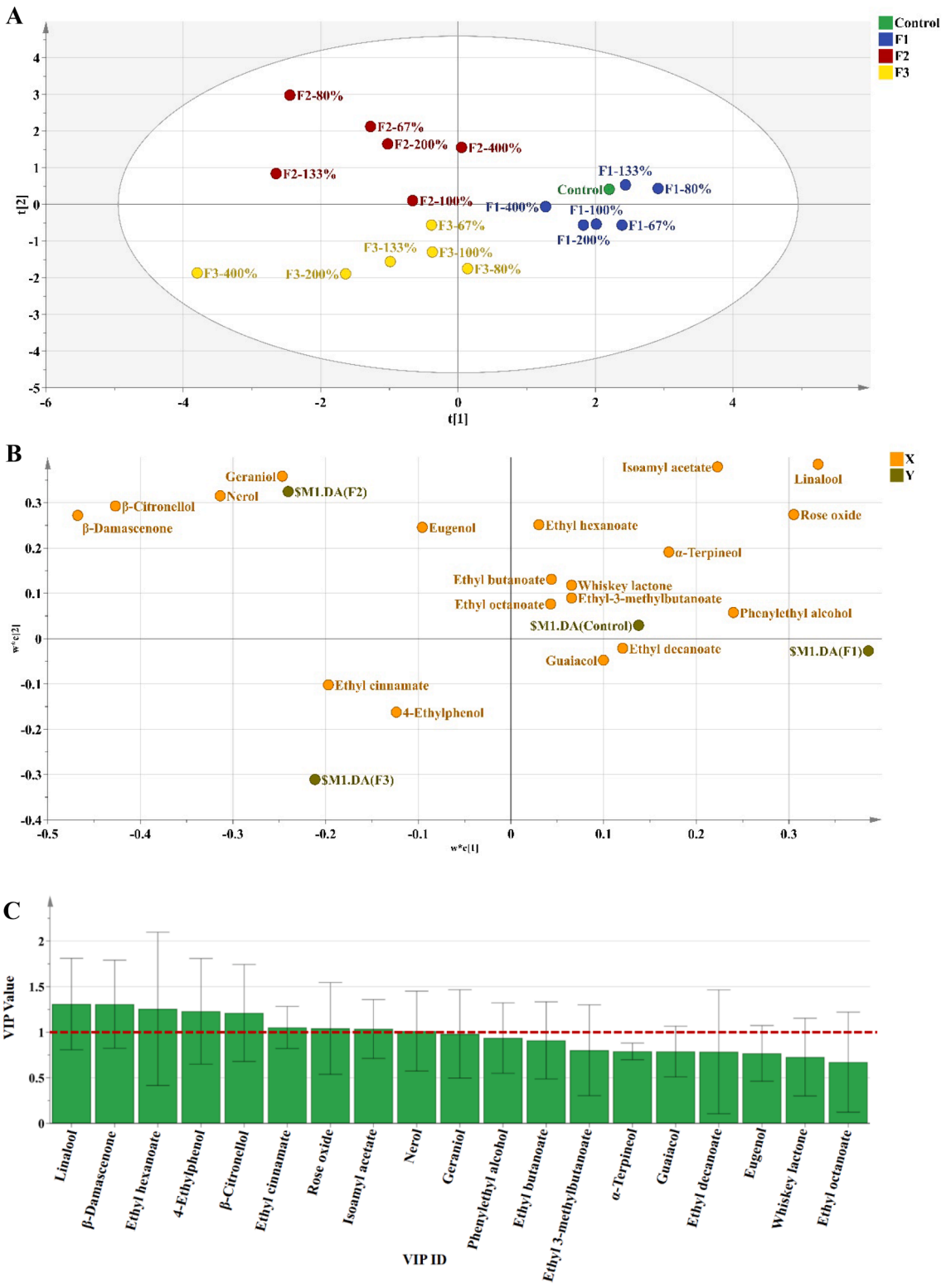


Fig. 3. Partial least squares discriminant analysis (PLS-DA) score plot (**A**), loading plot (**B**) and variables important in the projection (VIP) plot (**C**) for aroma compounds affected by different concentrations (67%, 80%, 100%, 133%, 200% and 400% of the original wine) of three phenolic fractions. Phenolic fractions: F1-phenolic acids, F2-monomeric/oligomeric procyanidins, F3-polymeric procyanidins. X and Y in the loading plot represent 19 aroma compounds and three phenolic fractions and control treatment.

increased in the presence of three phenolic fractions, whereas the volatility of aromas in cluster 2 mostly decreased, with the greatest decrease in the release of ethyl decanoate in cluster 3.

For aged aroma, whiskey lactone and guaiacol clustered into one group, then with eugenol, while 4-ethylphenol formed its own group (Fig. 2 C). It is noteworthy that the volatility of 4-ethylphenol tended to decrease at most concentrations of three phenolic fractions.

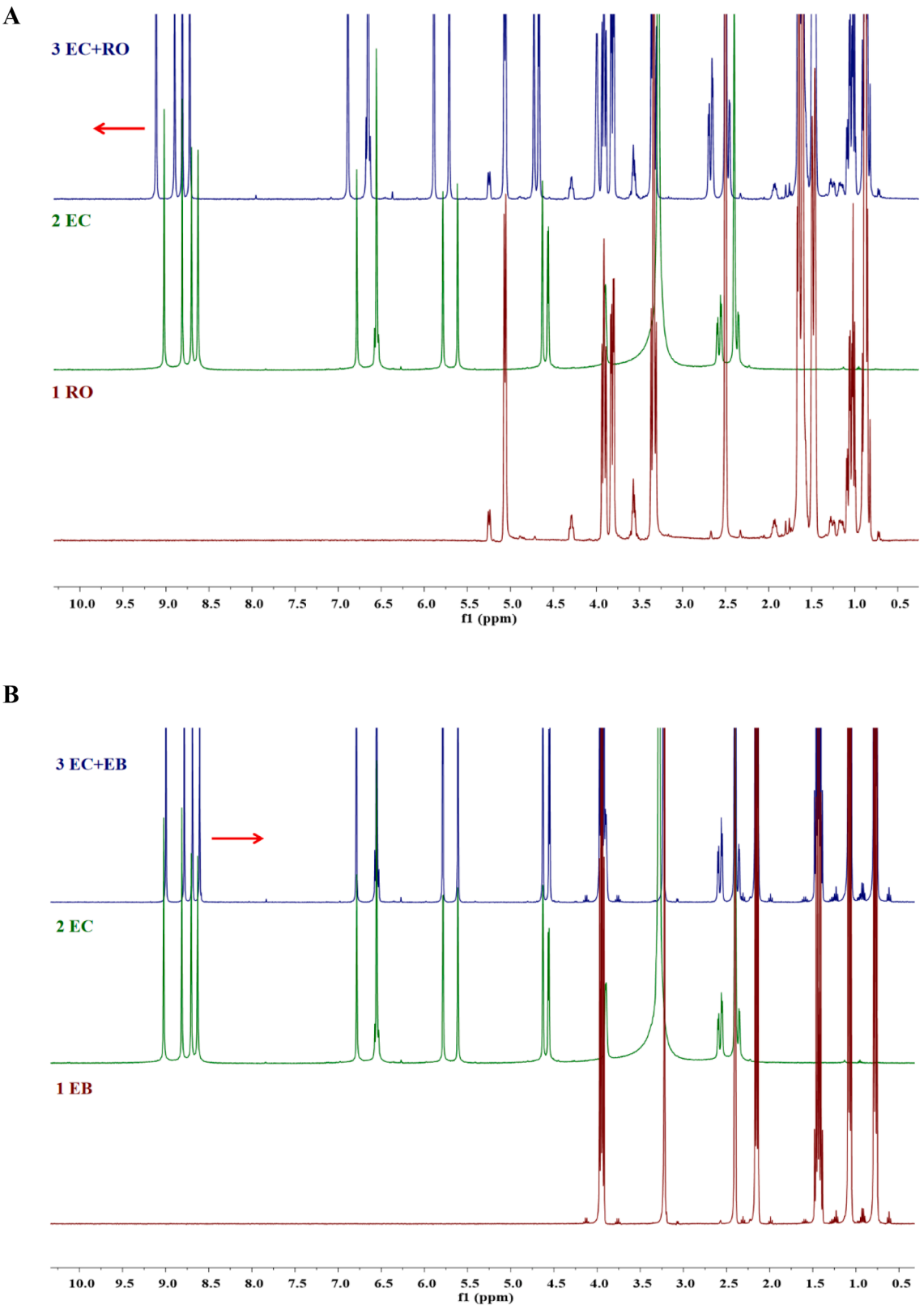


Fig. 4. ^{1}H NMR spectra of aroma compounds in the presence and absence of epicatechin. Aroma compounds include RO-rose oxide (**A**), EB-ethyl butanoate (**B**), WL-whiskey lactone (**C**). The abbreviation EC stands for epicatechin. Aroma compound (bottom), epicatechin (middle), aroma and epicatechin complexes (top).

PLS-Da

PLS-DA was used to further elucidate the relationship between different concentrations of phenolic fractions and the volatility of aroma compounds. The scoring plot shows that the different concentrations of phenolic fractions could be effectively discriminated (Fig. 3 A). All F1 samples clustered on the right side of the score plot, all F2 samples

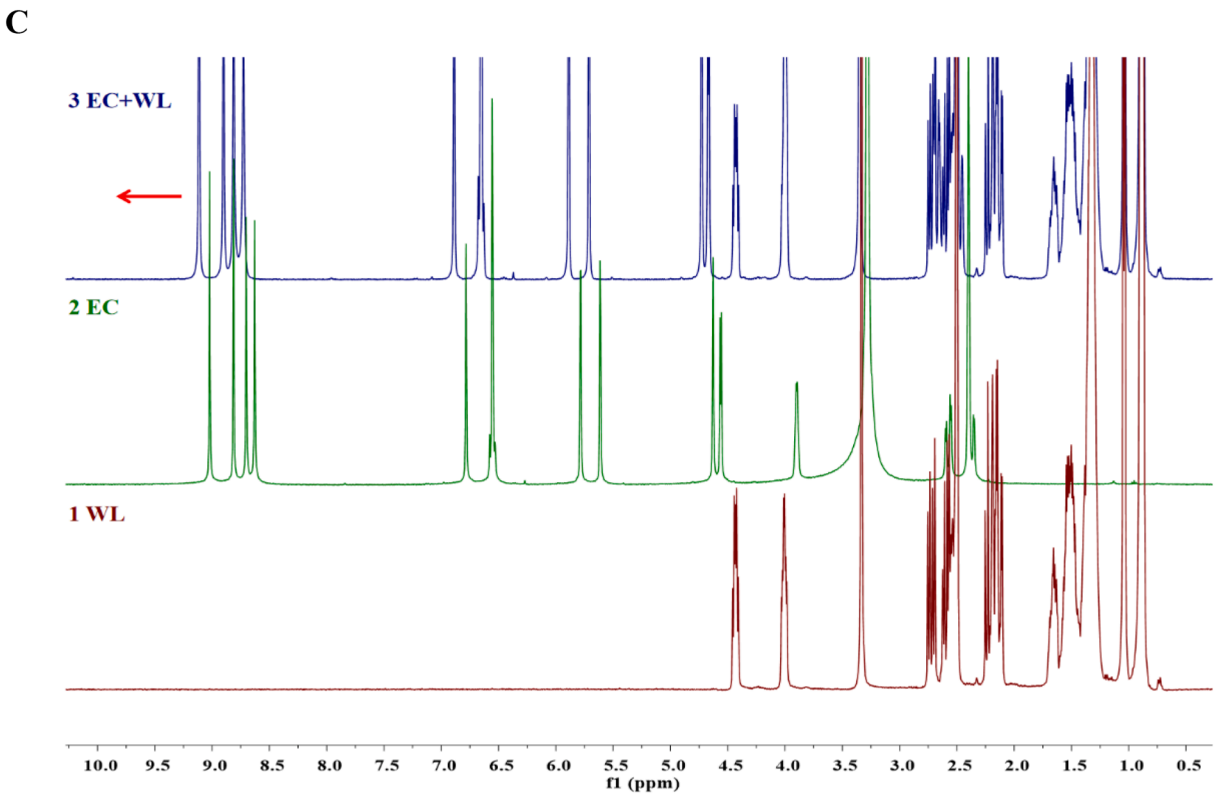


Fig. 4. (*continued*).

clustered in the upper-left quadrant and all F3 samples were in the lower left quadrant. The independent variable, dependent variable and prediction fit index for this model were R2X (cum) = 0.822, R2Y (cum) = 0.858, Q2 (cum) = 0.839, meaning that the model has high explanation and prediction ability.

The loading plot better illustrates the differences between aroma compounds according to the different concentrations of phenolic fractions (Fig. 3 B). Results revealed that F1 samples were highly correlated with some floral and fruity aromas such as linalool, isoamyl acetate, rose oxide, α-terpineol and phenylethyl alcohol. Equally, some floral aromas including geraniol, nerol, β-damascenone, β-citronellol were highly correlated with F2. Indeed, the volatility of these more hydrophobic (Log P > 3.30) floral aromas was found to be enhanced in the presence of F2 from the results of 3.2.1. In the case of F3, fruity aroma ethyl cinnamate and aged aroma 4-ethylphenol showed a certain correlation.

To further determine the key aroma compounds affected by different phenolic fractions, the variable important for the projection (VIP) plot of PLS-DA was used to quantify the contribution of the variables to the classification. In general, a VIP value > 1 is considered an important differential compound with a high contribution to the classification (Lenhardt, Bro, Zeković, Dramićanin, & Dramićanin, 2015). As shown in Fig. 3 C, 9 aroma compounds with a VIP value > 1 were important differential compounds, including 5 floral, 3 fruity and 1 aged aroma. These compounds were specifically linalool, β-damascenone, ethyl hexanoate, 4-ethylphenol, β-citronellol, ethyl cinnamate, rose oxide, isoamyl acetate and nerol. In wine tasting, they are described as important notes that can confer a wine floral, fruity, sweet, aging characteristics, adding complexity and intensity to the wine's aromas (Jeromel et al., 2019). The above results revealed that the influence of three phenolic fractions on the aroma volatility was different, depending on the structural properties and concentration for polyphenols and the physicochemical properties for aroma compounds.

Molecular mechanism of the matrix effect of phenolics on aroma compounds

Based on the above analysis, three aroma compounds (rose oxide, ethyl butanoate and whiskey lactone) and epicatechin, a major component of polyphenols, were selected to furtherly explain the matrix effect of phenolics at molecular level using HS-SPME-GC–MS and NMR technologies.

HS-SPME-GC–MS analysis

Table S2 shows that the headspace concentrations of all three aroma compounds were significantly decreased ($p < 0.05$) by adding epicatechin to the model wine. It implied that epicatechin has a retention effect on these aroma compounds, probably as a result of the interaction of aroma compounds with epicatechin.

^{1}H NMR analysis

The aroma-epicatechin interactions were studied by ^{1}H NMR spectroscopy, analyzing the changes in chemical shifts which described the chemical environment of a particular nuclei for aromas and epicatechin protons after their reactions (Hu, Xu, & Cheng, 2012). The chemical structure and chemical shifts of the aroma and epicatechin are shown in Table S3. The hydrogen spectra of rose oxides and epicatechin showed a significant variation in chemical shift after mixed (Fig. 4 A). The proton resonance signals of epicatechin were all systematically shifted to the low-frequency field (shifted to the left), which may be due to a decrease in the electron cloud density around epicatechin after mixing with rose oxide (deshielding effect) (Hu, Cheng, Ma, Wu, & Xu, 2009). These shifted hydrogen peaks were attributed to –OH and -H on the A, B and C rings of epicatechin. Conversely, the proton resonance signals at the a (δ = 5.06 ppm), d (δ = 3.57 ppm), f (δ = 1.62 ppm) and k (δ = 0.86 ppm) positions of rose oxide were shifted to the high-frequency field (shifted to the right), which may be associated with an increase in the electron cloud density (shielding effect). These four hydrogen peaks were

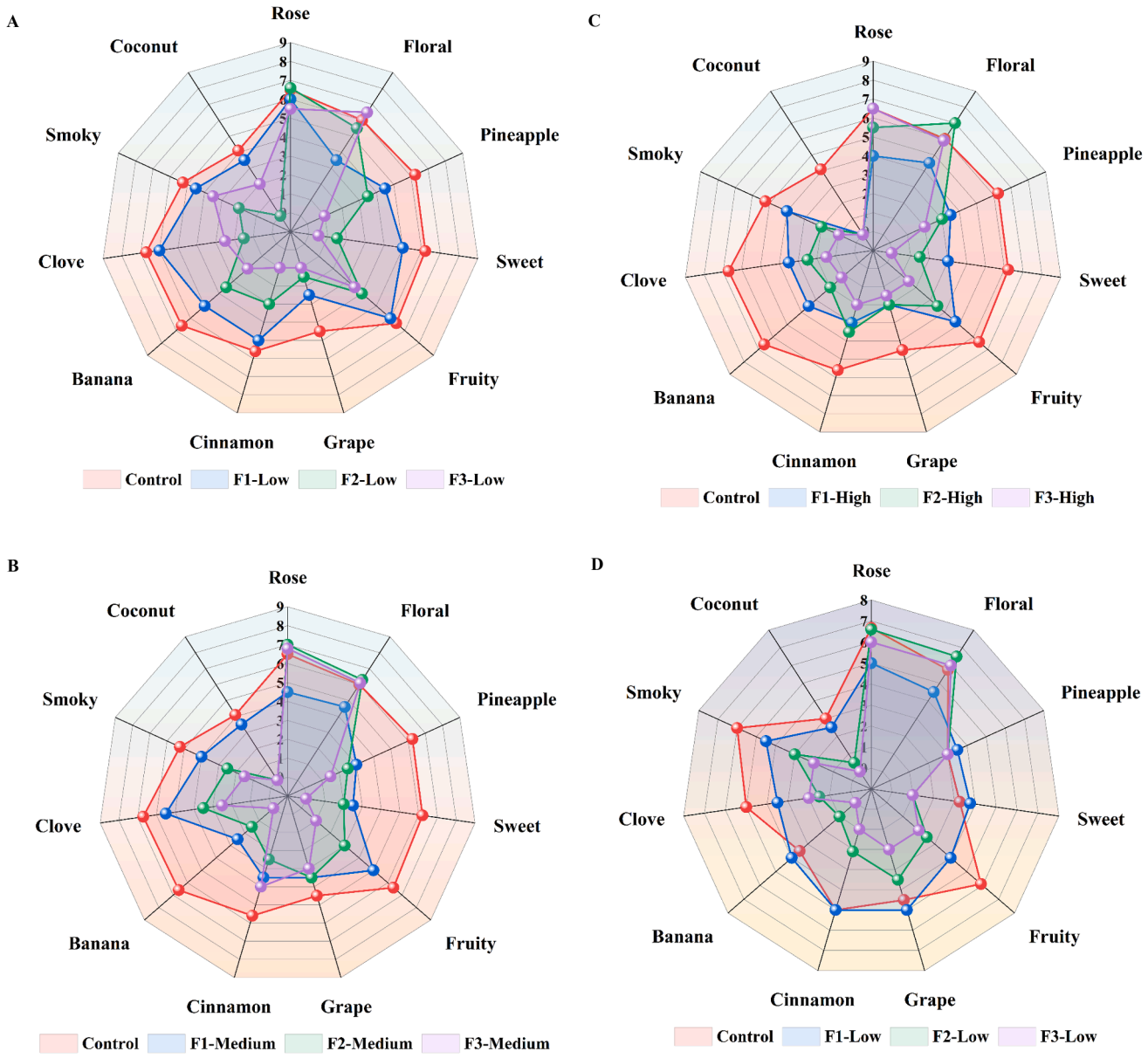
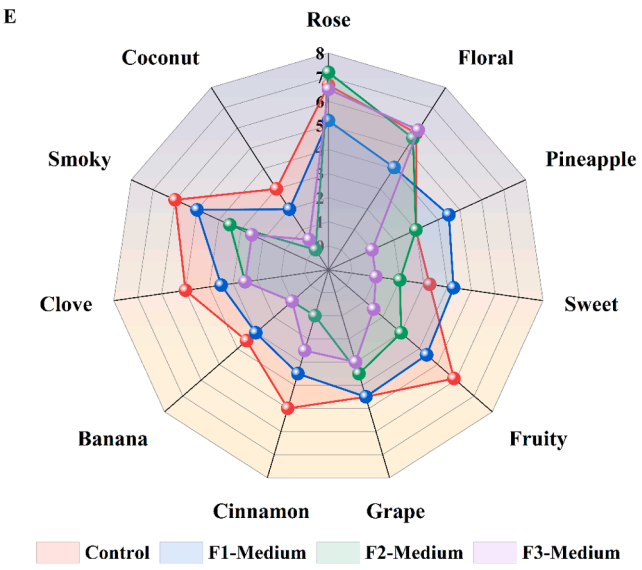


Fig. 5. Aroma profile in model wine and Pinot Noir affected by three phenolic fractions. **A-C** and **D-F** are experiments carried out in model wine and Pinot Noir, respectively. Low, medium, and high represent the different concentrations of phenolic fractions added to the wine. Phenolic fractions: F1-phenolic acids, F2-monomeric/oligomeric procyanidins, F3-polymeric procyanidins. Control: solution without phenolic fractions.

attributed to the -H around the double bond for rose oxide. With the addition of epicatechin, the polar group double bond may shift to the high-frequency field by π-π conjugation (Gigl, Hofmann, & Frank, 2021), allowing the protons on the more distant carbon to easily overlap with the hydrogen orbital and weaken the vibration. These chemical shift variations suggested that rose oxide could interact with epicatechin, and explained the previous results of reduced volatility due to the retention effect of polyphenolic matrix on rose oxide.

As shown in Fig. 4 B, the chemical shift behavior of both epicatechin and ethyl butanoate changed significantly when they were mixed. The resonance signals of the hydroxyl groups on the benzene rings of both epicatechin A and B ($\delta = 9.02$ ppm, 8.81 ppm, 8.70 ppm and 8.63 ppm) were systematically shifted towards the high-frequency field (shielding effect), which could be the consequence of their reaction with ethyl butanoate. The -H on the benzene ring of epicatechin C ($\delta = 4.56$ ppm) displayed a slight high-frequency shift in the proton resonance signal due to structural changes leading to an increase in the density of the electron cloud outside the hydrogen nucleus. In contrast, the protons at 5.78 ppm and 5.61 ppm were attributed to hydrogens on the B ring of epicatechin, whose resonance signal shifted to the low-frequency field with increased deshielding effects. The remaining hydrogen peak of epicatechin was not shifted, implying that the active site of epicatechin was mainly on the hydroxyl group. Indeed, the hydrogen protons on ethyl butanoate were more active and readily bound to the four hydroxyl groups on epicatechin, resulting in a slightly high-frequency field for the proton resonance signal, indicating a weakened intermolecular interaction between the epicatechin and ethyl butanoate.

The NMR spectrum of the reaction between whisky lactone with epicatechin exhibited significant chemical shift change in Fig. 4 C. The resonance signals of –OH and -H on the A and B rings of epicatechin and -H on the C ring ($\delta = 4.56$ ppm, 2.57 ppm, 2.40 ppm) were systematically low-frequency field shifted due to a decrease in the density of the outer electron cloud of these protons. Simultaneously, the proton resonance signal of the whisky lactone ($\delta = 4.00$–4.43 ppm, 2.59 ppm, 1.33



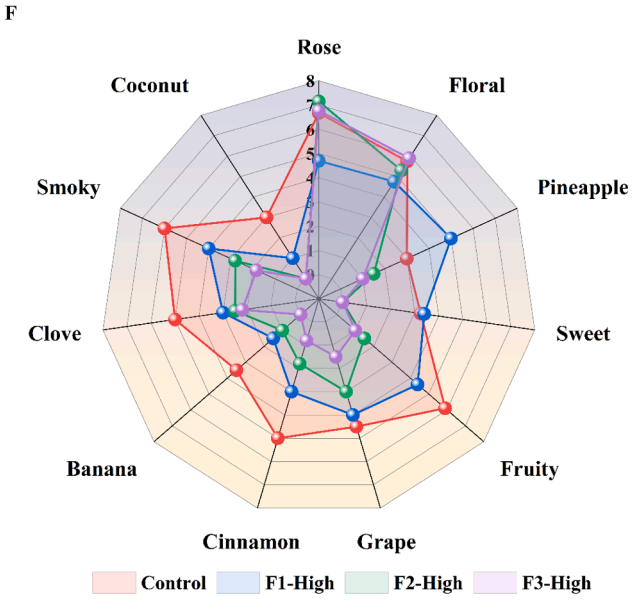


Fig. 5. (*continued*).

ppm) was also shifted towards the low-frequency field, with increased deshielding effects. In conclusion, the resonance signals of some protons from both whisky lactone and epicatechin were low-frequency field shifted, which may be due to the strong intermolecular forces between these molecules.

The NMR results explained the existence of interactions between three aroma compounds and epicatechin from a molecular point of view. This is consistent with the results of HS-SPME-GC–MS analysis in 3.4.1 and explained the fact that the volatility of these aromas from floral, fruity, and aged aroma was reduced in the presence of polyphenols in the previous experiments.

Sensory evolution

In model wine, the perception scores for each aroma attribute decreased to varying degrees after the addition of three phenolic fractions, apart from floral aroma attributes (Fig. 5 A-C). F2 and F3 exhibited stronger reductions in perceived score than F1, which is in accordance with the physicochemical analysis results in 3.2 that the effect of monomerics/oligomerics and polymerics on the release of aroma compounds were more readily determined than for phenolic acids. For three different types of aromas, only the perception scores for floral and rose attributes showed a slight increase in the presence of F2 or F3. This verified the physicochemical results that polymeric tannins promoted the release of certain floral aromas, whereas most fruity and aged aromas like pineapple, grape, sweet, cinnamon, clove, smoky, coconut and clove were retained in the phenolic matrix. Furthermore, the high concentration of phenolic fractions resulted in lower perceived scores for the fruity and aged aroma attributes than the low concentration of phenolic fractions. This is in agreement with previous work, where high concentrations of polyphenols reduced the volatility and perceived intensity of most aromas due to matrix effects (Goldner, Lira, van Baren, & Bandoni, 2011; Mitropoulou et al., 2011).

In the case of Pinot Noir, F2 and F3 exhibited a lower perceived intensity of most aroma attributes compared to F1, indicating that polymeric tannins could have a stronger retention effect than phenolic acids (Fig. 5 D-F). The perceived scores for some aromas in the presence of F1 were slightly higher than the control, with different sensory results from

model wine, which may be related to differences in wine systems. Additionally, the sensory scores for the floral aroma attributes were higher than the fruity and aged aroma attributes after adding phenolic fractions, especially F2 and F3, in agreement with the above experiment results. As the concentration of the phenolic fraction increased, the perceived scores for most aroma attributes were found to follow a decreasing trend.

Conclusion

The impact of three phenolic fractions on the volatility of floral, fruity, and aged aromas was different according to the structural properties of polyphenols and aroma compounds. In the model wine matrix, all three phenolic fractions displayed retention effects on most fruity and aged aromas. Particularly, monomerics/oligomerics and polymerics decreased the volatility of more fruity aromas than phenolic acids, and polymeric procyanidins at high concentrations showed the strongest matrix effect on fruity aromas. Moreover, hydrophobicity was an essential factor influencing the interaction of floral and fruity aromas with phenolic fractions. Monomerics/oligomerics and polymerics promoted the release of high hydrophobic (Log P $>$ 3.30) floral aromas, whereas the less hydrophobic (Log P $<$ 2.85) fruity aromas were retained in three phenolic fractions matrix. Chemical shift changes determined by NMR confirmed that the decrease in the headspace levels of rose oxides, ethyl butanoate and whisky lactone was attributed to interactions with epicatechin. Sensory analysis revealed that the presence of three phenolic fractions in model wine and Pinot Noir reduced the perceived intensity of fruity and aged aromas, especially at higher concentrations and polymerization of phenolic fractions. This study provided information on the volatility of wine aromas affected by polyphenols with different structural properties during wine consumption. This work also provides clues for enologist to effectively modulate the overall feature of wine by designing winemaking techniques such reconstituting the phenolic fractions.

CRediT authorship contribution statement

Shengnan Wang: Writing – original draft, Formal analysis, Data curation, Software. **Qianting Zhang:** Methodology, Investigation, Data curation. **Pengtao Zhao:** Conceptualization, Resources, Writing – review & editing, Funding acquisition. **Zeqiang Ma:** Data curation, Validation, Software. **Junxiang Zhang:** Resources, Investigation. **Wen Ma:** Resources, Supervision. **Xiaoyu Wang:** Resources, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The research was supported by the Science and Technology Department of Shaanxi Province (2020ZDLNY05-08) and Key Research and Development Program of Xianyang (2021ZDYF-NY-0021).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2022.100281.

References

Aronson, J., & Ebeler, S. E. (2004). Effect of polyphenol compounds on the headspace volatility of flavors. *American Journal of Enology and Viticulture, 55*(1), 13–21.

Basalekou, M., Kyraleou, M., Pappas, C., Tarantilis, P., Kotseridis, Y., & Kallithraka, S. (2019). Proanthocyanidin content as an astringency estimation tool and maturation index in red and white winemaking technology. *Food Chemistry, 299*, Article 125135.

Cameleyre, M., Lytra, G., & Barbe, J. C. (2018). Static headspace analysis using low-pressure gas chromatography and mass spectrometry, application to determining multiple partition coefficients: A practical tool for understanding red wine fruity volatile perception and the sensory impact of higher alcohols. *Analytical Chemistry, 90*(18), 10812–10818.

Carpena, M., Fraga-Corral, M., Otero, P., Nogueira, R. A., Garcia-Oliveira, P., Prieto, M. A., & Simal-Gandara, J. (2020). Secondary aroma: Influence of wine microorganisms in their aroma profile. *Foods, 10*(1), 51.

De Castilhos, M. B. M., Del Bianchi, V. L., Gómez-Alonso, S., García-Romero, E., & Hermosín-Gutiérrez, I. (2019). Sensory descriptive and comprehensive GC–MS as suitable tools to characterize the effects of alternative winemaking procedures on wine aroma. Part I: BRS Carmem and BRS Violeta. *Food Chemistry, 272*, 462–470.

De Rosso, M., Panighel, A., Dalla Vedova, A., Stella, L., & Flamini, R. (2009). Changes in chemical composition of a red wine aged in acacia, cherry, chestnut, mulberry, and oak wood barrels. *Journal of Agricultural and Food Chemistry, 57*(5), 1915–1920.

Dufour, C., & Bayonove, C. L. (1999). Interactions between wine polyphenols and aroma substances. An insight at the molecular level. *Journal of Agricultural and Food Chemistry, 47*(2), 678–684.

Ferreira, V., López, R., & Cacho, J. F. (2000). Quantitative determination of the odorants of young red wines from different grape varieties. *Journal of the Science of Food and Agriculture, 80*(11), 1659–1667.

Flanzy, C. (2003). *Enologia Fundamentos Cientificosy Tecnológicos* (2nd ed., pp. 137–168). Madrid, Spain: AMV-Mundi Prensa.

Gigl, M., Hofmann, T., & Frank, O. (2021). NMR-based studies on odorant-melanoidin interactions in coffee beverages. *Journal of Agricultural and Food Chemistry*.

Goldner, M. C., Lira, P. D., van Baren, C., & Bandoni, A. (2011). Influence of polyphenol levels on the perception of aroma in Vitis vinifera cv. Malbec wine. *South African Journal of Enology and Viticulture, 32*(1), 21–27.

Guth, H. (1997). Quantitation and sensory studies of character impact odorants of different white wine varieties. *Journal of Agricultural and Food Chemistry, 45*(8), 3027–3032.

Hu, J., Cheng, Y., Ma, Y., Wu, Q., & Xu, T. (2009). Host−guest chemistry and physicochemical properties of the dendrimer−mycophenolic acid complex. *The Journal of Physical Chemistry B, 113*(1), 64–74.

Hu, J., Xu, T., & Cheng, Y. (2012). NMR Insights into Dendrimer-based host–guest systems. *Chemical Reviews, 112*(7), 3856–3891.

Jeromel, A., Korenika, A. M. J., & Tomaz, I. (2019). 6 - An influence of different yeast species on wine aroma composition. In A. M. Grumezescu, & A. M. Holban (Eds.), *Fermented beverages* (pp. 171–285). Woodhead Publishing.

Jung, D. M., Ropp, J. S. D., & 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.

Jung, D. M., & Ebeler, S. E. (2003). Headspace solid-phase microextraction method for the study of the volatility of selected flavor compounds. *Journal of Agricultural and Food Chemistry, 51*(1), 200–205.

Lenhardt, L., Bro, R., Zeković, I., Dramićanin, T., & Dramićanin, M. D. (2015). Fluorescence spectroscopy coupled with PARAFAC and PLS DA for characterization and classification of honey. *Food Chemistry, 175*, 284–291.

Li, Y. G., Tanner, G., & Larkin, P. (1996). The DMACA–HCl protocol and the threshold proanthocyanidin content for bloat safety in forage legumes. *Journal of the Science of Food and Agriculture, 70*, 89–101.

Longo, R., Carew, A., Sawyer, S., Kemp, B., & Kerslake, F. (2021). A review on the aroma composition of Vitis vinifera L. Pinot noir wines: Origins and influencing factors. *Critical Reviews in Food Science and Nutrition, 61*(10), 1589–1604.

López, R., Aznar, M., Cacho, J., & Ferreira, V. (2002). Determination of minor and trace volatile compounds in wine by solid-phase extraction and gas chromatography with mass spectrometric detection. *Journal Of Chromatography A, 966*, 167–177.

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), 76–82.

Mateo, J. J., & Jiménez, M. (2000). Monoterpenes in grape juice and wines. *Journal of Chromatography A, 881*(1), 557–567.

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.

Moreau, C., & Guichard, E. (2006). Flavor-food compound interactions by NMR spectroscopy. In G. A. Webb (Ed.), *Modern magnetic resonance* (pp. 1611–1615). Dordrecht: Springer.

Muñoz-González, C., Martín-Álvarez, P. J., Moreno-Arribas, M. V., & Pozo-Bayón, M. (2014). Impact of the nonvolatile wine matrix composition on the in vivo aroma release from wines. *Journal of Agricultural and Food Chemistry, 62*(1), 66–73.

Perez-Jiménez, M., Chaya, C., & Pozo-Bayón, M.Á. (2019). Individual differences and effect of phenolic compounds in the immediate and prolonged in-mouth aroma release and retronasal aroma intensity during wine tasting. *Food Chemistry, 285*, 147–155.

Petrozziello, M., Asproudi, A., Guaita, M., Borsa, D., Motta, S., Panero, L., & Bosso, A. (2014). Influence of the matrix composition on the volatility and sensory perception of 4-ethylphenol and 4-ethylguaiacol in model wine solutions. *Food Chemistry, 149*, 197–202.

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*, 1157.

Pozo-Bayón, M., & 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). New York: Springer Science + Business Media.

Riou, V., Vernhet, A., Doco, T., & Moutounet, M. (2002). Aggregation of grape seed tannins in model wine—effect of wine polysaccharides. *Food Hydrocolloids, 16*(1), 17–23.

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.

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology, 299*, 152–178.

Sun, B., Leandro, C., Ricardo, d. S., & Spranger, I. (1998). Separation of grape and wine proanthocyanidins according to their degree of polymerization. *Journal of Agricultural and Food Chemistry, 46* (4), 1390–1396.

Tetik, M. A., Sevindik, O., Kelebek, H., & Selli, S. (2018). Screening of key odorants and anthocyanin compounds of cv. Okuzgozu (Vitis vinifera L.) red wines with a free run and pressed pomace using GC-MS-Olfactometry and LC-MS-MS. *Journal of Mass Spectrometry, 53*(5), 444–454.

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.

Wang, S. N., Wang, X. Y., Zhao, P. T., Ma, Z. Q., Zhao, Q. H., Cao, X. M., … Du, G. R. (2021). Mannoproteins interfering wine astringency by modulating the reaction between phenolic fractions and protein in a model wine system. *LWT - Food Science and Technology, 152*, Article 112217.

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*, Article 128288.

Zhang, Q., Chen, T., Wang, X., Zhao, P., Lei, X., Liu, P., … Guo, Y. (2020). Influence of simulated grape crushing process on phenolic compounds extraction, astringency and color of Cabernet Sauvignon model wine. *LWT - Food Science and Technology, 128*, Article 109514.

Zhao, P., Qian, Y., He, F., Li, H., & Qian, M. (2017). Comparative characterization of aroma compounds in merlot wine by Lichrolut-En-based aroma extract dilution analysis and odor activity value. *Chemosensory Perception, 10*(4), 149–160.