

GAS CHROMATOGRAPHY COUPLED WITH MASS SPECTROMETRY DETECTION FOR THE VOLATILE PROFILING OF *VITIS VINIFERA* CV. CARMÉNÈRE WINES

ANA MARÍA DOMÍNGUEZ* AND EDUARDO AGOSIN^{1,2}

¹Centro de Aromas, DICTUC SA, and ²Department of Chemical and Bioprocess Engineering, School of Engineering, Pontificia Universidad Católica de Chile, Av. Vicuña Mackenna 4860, Macul, Santiago, CHILE.

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ABSTRACT

The volatile metabolome of *Vitis vinifera* C.V. Carménère wines, a unique Chilean winegrape variety, was characterized in this work. After solvent extraction with dichloromethane (LLE) or head space solid phase microextraction (HS-SPME), the resulting volatile compounds were analyzed by gas chromatography-mass spectrometry (GC-MS). Specific methods were carried out too for the determination of methoxyppyrazines and thiols. Almost 150 compounds corresponding to aliphatic and aromatic alcohols, organic acids, acetate and ethyl esters, lactones, terpenes, norisoprenoids, pyrazines, thiols and phenolic compounds were found in these wines. Both extraction techniques were complementary; HS-SPME allowed to identify several important compounds that were not detected by liquid-liquid solvent extraction, in particular b-damascenone, nerolidol, citronellol, linalool, ethyl 2-methyl-butylate and geranyl acetone. Several varietal volatile compounds with significant odorant properties were also identified: 3-mercaptopentyl acetate (3MHA), 3-mercaptopentyl-1-ol (3MH), benzenemethanethiol (BM), 2-furanmethanethiol (2FM); and 2-isobutyl-3-methoxyppyrazine (IBMP) while 2-isopropyl-3-methoxyppyrazine (IPMP) and 4-mercapto-4-methylpentan-2-one (4MMP) were not detected. Twenty two of these compounds, including b-damascenone, several thiols, ethyl octanoate, 2-phenylethanol, and 2-isobutyl-3-methoxyppyrazine, showed significant odor activities values (OAV), and were clearly related with the cooked fruits, berries-like and herbaceous notes characteristic of Carménère wines. Olfactometric techniques are underway to validate the sensory impact of these compounds.

INTRODUCTION

Wine quality and identity are the result of several interacting factors, particularly terroir and grape variety, but also viticultural and winemaking practices. Wine aroma is a key component of the former. It is composed by several hundred of volatile compounds that belong to different chemical families, arising from the grape metabolism, the yeast fermentation and the aging process. Several studies have already recognized the close relationship between the varietal, differential character of a wine and the grapes from which it is produced. Many of these compounds - terpenes, sesquiterpenes, norisoprenoids, benzene derivatives, aromatic alcohols and C6 alcohols - are able to break the common "vinous matrix" present in any wine, to express the unique features of the variety in the wine¹⁻⁴.

Some important compounds of the wine aroma include: C6-compounds like 1-hexanol, (E)-3-hexenol and (Z)-3-hexenol, responsible for some green notes, normally released during prefermentative operations^{5,6}; higher alcohols, fatty acids and esters produced during yeast fermentation^{7,8}; key aromatic varietal compounds such as monoterpenes, norisoprenoids and thiols mostly released during fermentation and aging, from their conjugated forms by chemical or enzymatic hydrolysis⁹⁻¹¹; and unique varietal compounds, like rotundone in Shiraz¹², fureanol in rosé wines¹³, cis-rose oxide in Gewürztraminer, 4-methyl-4-mercaptopentane in Sauvignon Blanc¹⁴ or methoxyppyrazines in Sauvignon and Carménère varieties^{15,16}.

These volatile components belong to many chemical families and show specific features, such as different polarity, solubility, volatility, stability, oxidation and degradation, among others. Thus, several extraction techniques have been employed to ensure full characterization of the volatile profile of grapes and wines, like solid-phase extraction (SPE)¹⁷⁻¹⁹; stir bar sorptive extraction (SBSE)^{20,21}, solvent-assisted flavor evaporation (SAFE)^{22,23}; dynamic headspace sampling^{24,25}; and liquid extraction with organic solvents (LLE)²⁶⁻²⁸. Although the latter is time consuming and could involve contamination with solvents, losses during the final concentration steps as well as artifact generation, it is still the most widely used extractive technique. A more recent, increasingly popular technique is solid-phase microextraction (SPME), which is based on the partitioning of analytes between the sample matrix and the extracting phase coating. The latter can be used for analyte concentration either by submersion in the liquid phase or by exposure to the gaseous phase in the headspace (HS-SPME). The sorbed analytes are thermally desorbed in a conventional GC injection port. This is a simple, fast, inexpensive, sensitive and solvent-free methodology²⁹⁻³².

Vitis vinifera cv. Carménère, is a red grape variety originating from Bordeaux, France, that was believed to be extinguished after the phylloxera plague that ravaged the french vineyard in the mid 19th century. However, in

1994, the variety was re-discovered in Chile, mostly mixed with Merlot vines. Carménère is currently the Chilean flagship variety with more than 7,000 hectares planted in most of the valleys of the country. This variety has adapted particularly well to the Chilean climate, soil and geographical conditions. Quantitative descriptive analyses of Carménère wines showed two major groups of descriptors associated with green, herbaceous notes on one side; and fruity, spicy, berry like notes on the other³³. Belancic and Agosin¹⁶ recently demonstrated the importance of methoxyppyrazines in relation with the strong vegetative aroma.

In this work, was studied the volatile chemical profiling from two regions of Chile monovarietal Carménère wines. The compounds present in high concentrations (major compounds) were extracted using different extraction methods: LLE and HS-SPME. Also, specific extraction techniques were employed for the isolation and quantitation of volatile compounds present at trace levels (minor compounds), *i.e.* pyrazines and thiols. Identification of several high impact odorants of Carménère wines and their correlation with known sensory descriptors of the variety was also attempted.

Experimental part

Materials

The two Carménère wines employed in this study were kindly donated, between the years 2007 and 2009, by Perez Cruz and Casa Silva wineries, situated in Maipo and Colchagua valleys, respectively. They were stored in the bottles at 15°C until their analysis. These, 2007, 2008 and 2009 wines were selected by the winemakers for their tipicity. The six Carménère wines samples were analyzed individually, in triplicate. Results shown in Table 1 correspond to the average data from all the Carménère wines analyzed, because their volatile profile was quite similar.

Dichloromethane, ethyl acetate, sodium p-hydroxymercurybenzoate, cysteine monohydrate hydrochloride, anhydrous sodium sulfate, 4-nonanol (internal standard) and DOWEX 1 resin were purchased from Merck (Darmstadt, Germany). Water was purified with a MilliQ system from Millipore (Bedford, MA). Nitrogen and helium gases were supplied by Indura (Santiago Chile). Deuterated 2-isobutyl-3-methoxyppyrazine (d₅-IBMP) used as internal standard, 2-isobutyl-3-methoxyppyrazine and 2-isopropyl-3-methoxyppyrazine were purchased by Sigma-Aldrich. Chemos (Germany) provided the thiols: heptanethiol (internal standard), 4-mercapto-4-methylpentan-2-one, 3-mercaptopentyl-1-ol, 3-mercaptopentyl acetate, 2-furanmethanethiol and benzenemethanethiol. Three SPME fibers of different polarity from Supelco, Sigma-Aldrich: 100µm Polydimethylsiloxane (PDMS), 50/30µm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) and 85µm Polyacrylate (PA), were used to provide complementary information about volatile profile of Carménère wines.

Liquid Liquid Extraction (LLE)

100 ml of wine, spiked with 354 µg of 4-nonanol as internal standard was extracted twice with 25 ml of dichloromethane at 4°C under nitrogen atmosphere. The sample was stirred for 30 min and then centrifuged at 5°C and 5000 rpm. The organic phases were combined and concentrated to 1 mL in a Vigreux column; then, the extract was gently reduced to 350 µL with nitrogen gas. 2 µL of this concentrated wine organic extract was employed for GC/MS analysis. All the analyses were done in triplicate.

HS-SPME extraction

20 mL of wine at a 1:1 dilution with milliQ water were placed in a 40 mL volume vial; after the addition of the internal standard and 1g of NaCl the vial was sealed with a Teflon septum and equilibrated at 40°C for 5 min with agitation. The fiber was then introduced and exposed to the bottle head space during 1 hour, keeping the agitation and vial temperature. After this period, the fibre was retracted and the adsorbed volatile compounds were thermally desorbed for 20 min at the injection port of the GC/MS equipment.

Minor compounds

Pyrazines extraction and quantitation was carried out in triplicate using the method reported by Belancic¹⁶.

Thiols were isolated and extracted using DOWEX anionic exchange resins as reported by Tominaga³⁴.

GC/MS analysis

A GC/MS Hewlett Packard 6890 gas chromatograph (GC) coupled to a HP 5973 mass selective detector (MSD) was used for the analyses. The analytes were separated on a DB Wax capillary column (60m x 0,25mm x 0,25 µm), applying the following temperature program: 40°C for 5 min; from 40°C to 240°C, temperature was raised at 3°/min and then holding for 10 min. The transfer line was settled at 250°C and mass detector conditions were: scan mode with electronic impact (EI) at 70 eV and source temperature of 150°C. Ultrapurified helium was used as the carrier gas with a nominal flow rate of 1.5 ml/min. The injections were carried out in splitless mode, setting the injector temperature for LLE at 180°C and for SPME at 250°C. Identification of the volatile components was achieved by comparing the mass spectra with the data system library (NIST/EPA/NIH). All compounds were quantified as 4-nonanol equivalents. All the data showed a coefficient of variation lower than 15%.

RESULTS AND DISCUSSION**Analysis of Carménère wines volatiles extracted with organic solvent (LLE)**

The GC/MS analysis of the Carménère Chilean wines revealed the complex chemical profile of this kind of matrix (Table 1) with the presence of several compounds belonging to different chemical families, like alcohols, esters, carbonyl compounds, acids, furfural and shikimic derivatives, among others. As usual for most alcoholic beverages^{8, 19, 35}, the most quantitatively significant volatile compounds in these wines corresponded to yeast-derived fermentative alcohols and esters. Alcohols represented 52% of the total volatile composition, followed by the esters (32%). The former group - 21 volatile compounds - is mainly composed by isoamyl alcohol (fusel alcohol note) and 2-phenylethanol (roses, pollen, flowery notes). Esters represented the highest number of volatiles identified (34 compounds). These compounds are mainly responsible for sweet, fruity and floral sensory notes in wines. Monoethyl succinate, ethyl lactate and diethyl succinate showed the highest concentrations. Ethyl 3-hydroxy-butyrate was present at a concentration of 206 µg L⁻¹, in average. The latter compound has been reported earlier as a possible contributor of strawberry and burnt marshmallow notes in Pinot Noir wines, as pointed out in Ugliano et al.³⁶

The C6 derivative compounds, 1-hexanol, (Z)-3-hexenol, (E)-3-hexenol, and (E)-2-hexenol represent, in average, only 0,5% of the total volatile composition. These volatiles are formed via lipoxygenase activity of the grape during pre-fermentative steps including harvesting, crushing, pressing and grape maceration^{6, 37}. Both Carménère wines showed a similar proportion of these compounds, with major quantity of 1-hexanol followed by E-3-hexenol.

Fermentative fatty acids are responsible for sour, mild, rancid and cheesy notes in wines. They were represented as 7% of the total wine volatile composition of Carménère wines, with acetic, octanoic and hexanoic acids being the major quantitative contributors.

Carbonyl volatile compounds, equivalent to 1% of the total volatile concentration, are formed as byproducts of microbial fermentation and chemical oxidation or from oak-barrels during winemaking and aging

³⁸. Acetoin and 2, 3-butanedione (diacetyl) were the major quantitative contributors in both wines. Acetoin is formed by the reduction of diacetyl; the latter is an intermediate in the decarboxylative reduction of pyruvic acid to 2, 3-butanediol. These volatile compounds give to wines buttery or butterscotch, nutty, and also sweet, caramel attributes^{39, 40}.

The origin of sulfur compounds in wines involves physical and microbial reduction reactions⁴¹. In Carménère wines, these compounds reached 0,4% of the total volatile composition. Sulfur compounds are mostly responsible for the production of unpleasant or reduced flavours in wines like cabbage, cooked vegetable, onion and garlic. 3-methylthio-1-propanol (methionol) was the dominant sulfur volatile compound in the wines, reaching a concentration of 396 µg L⁻¹. This methionine derived compound has been reported to vary largely, according to the grape variety; if present in sufficiently high concentration, it gives a potato-, meat-like note to wines. It has been reported in wines at concentrations between 145 and 2000 µg L⁻¹^{36, 42, 43}.

Volatile compounds such as lactones, volatile phenols, shikimic acid derivatives and furfural derivatives migrate from oak wood to wine during maceration with oak chips or aging, playing an important role in wine quality^{44 - 46}. In Carménère wines, this group of substances represents 6,2% of the total volatile composition, with γ -butyrolactone, furfural and 4-carbomethoxy- γ -butyrolactone as the major contributors.

Terpenes and norisoprenoids are varietal components which play an important role in the flavour of wines. Their content is mostly related with the grape variety and viticultural factors (terroir, climate, water retention capacity, sun exposition, irrigation treatment, etc.). Terpenes could be found in grapes as sugar-conjugated, odourless precursors or as free volatiles. The most common representatives of this family are linalool, geraniol, nerol, linalool oxides, α -terpineol and nerol oxide. Norisoprenoids are formed by direct, oxidative degradation of carotenoids, such as β -carotene or lycopene, and can be stored as glycoconjugates, too. The terpenes and norisoprenoids in their odorant form can be further released from their glycoside precursors through acid or enzymatic hydrolysis during fermentation and aging. Norisoprenoids have very low olfactory perception thresholds and so, they have a high sensorial impact on wine aroma^{47, 48}. In the solvent-extracted Carménère wines, only two norisoprenoids were found, i.e. iridomyrmecin - in low concentration and only for Colchagua valley - and 3-oxo- α -ionol which contributes with honey and tobacco notes. An increase in the concentration of these compounds, as well as the release of other norisoprenoids, such as α - and β -ionol and their derivatives, 3-hydroxy-damascone, vitispiranes, vomifoliol, etc. during Carménère aging was recently demonstrated^{49, 50}.

Table 1. Average concentration of free volatile compounds found in young Carménère wines using LLE and HS-SPME with PDMS, DVB/CAR/PDMS and PA fibers.

Compound	LLE	HS-SPME		
	(µg L ⁻¹)	PDMS	DVB/ CAR/ PDMS	PA
Carbonyl compounds				
acetaldehyde		6		
2,3-butanedione	722	28	9	13
2,3-pentanedione	109	3	2	
acetoin	1096	4		
3-methyl-3-buten-2-one	10			
4-nonanone		12	19	
3-ethyl-4-heptanone	9		28	
2-hydroxy-pentan-3-one	72			
4-hydroxy-4-methyl-2-pentanone	35			
benzaldehyde	8			
2-octanone	9			
1-hydroxy-2-propanone	28 ^a			
3-hydroxy-4-phenyl-2-butanone	41 ^a			
Total average	2139	56	59	13

DVB/CAR/PDMS

Compound	LLE ($\mu\text{g L}^{-1}$)	HS-SPME ($\mu\text{g L}^{-1}$)		
		PDMS	DVB/ CAR/ PDMS	PA
Alcohols Cont.				
n-propanol	313	19	13	35
isobutanol	2768	44	80	87
1-butanol	264			
isoamyl alcohol	45460	1634	1310	2235
3-methyl-3-buten-1-ol +	43			
1-pentanol				
4-methyl-1-pentanol	40			
4-heptanol	10	4	4	
2-heptanol	5			
1-Heptanol	58			
3-methyl-1-pentanol	81			
3-ethoxy-1-propanol	158			
2,3-butanediol	5492	28		23
1,3-butanediol	1165			
2-(2-butoxy-ethoxy ethanol)	36			
benzyl alcohol	191			
2-phenylethanol	19937	122	235	627
3-octanol	10	17	20	13
2,6-dimethyl-4-heptanol	164 ^b			
3-ethyl-4-heptanol		12	13	
octanol	25	7	9	10
6-undecanol		129	112	90
1-decanol			7	
1-dodecanol		25	2	
3-methyl-3-buten-2-ol	200 ^a			
2-methyl-3-buten-2-ol	32 ^a			
Total average	76452	312	398	740
C6 Compounds				
1-hexanol	615	50	52	64
E-3-hexen-1-ol	51			
Z-3-hexen-1-ol	20			
Z-2-hexen-1-ol	7			
Total average	693	50	52	64
Acids				
acetic acid	4990	66	41	
propanoic acid	29			
isobutyric acid	312			
butyric acid	214			
valeric acid	1027			
hexanoic acid	1076	10	24	
2-hexenoic acid (isom.)	14			
heptanoic acid	10			
octanoic acid	1656	157	147	
decanoic acid	354	95	26	
hexadecanoic acid	102			
phenyl acetic acid	104			
Total average	9888	329	238	
Terpenes and norisoprenoids				
3-oxo-a-ionol	25			
iridomyrcin	6 ^b			
tyrosol	1043			
linalool		6	7	
b-citroneroll		12	14	

Compound	LLE ($\mu\text{g L}^{-1}$)	HS-SPME ($\mu\text{g L}^{-1}$)		
		PDMS	DVB/ CAR/ PDMS	PA
b-damascenone		30	22	
geranyl acetone		11	4	
nerolidol		34	8	24
Total average	1074	93	55	24
Esters Cont.				
methyl acetate			5	
ethyl acetate		720	707	623
ethyl propanoate	73			
ethyl isobutyrate	18	12	4	
propyl acetate	27	4	3	
isobutyl acetate	43	7	6	
ethyl butyrate	120	46	31	12
ethyl -2-methyl-butyrate		6	4	
ethyl isovalerate	9	8	6	
butyl acetate	9 ^b			
isoamyl acetate	908	742	579	287
ethyl hexanoate	267	662	524	206
hexyl acetate	46	31	27	
ethyl E-2-hexenoate		8		
ethyl lactate	12533	38	38	54
ethyl-2-hydroxy butyrate	5			
methyl octanoate		8	8	
ethyl octanoate	499	4144	3456	1790
ethyl-3-hydroxybutyrate	206			
isoamyl hexanoate			15	
2,3-butanediol monoacetate	171			
ethyl nonanoate		27	22	10
methyl decanoate			4	
ethyl decanoate	129 ^b	1688	1018	989
isoamyl octanoate		51	32	27
diethyl succinate	7114	285	317	339
1,3-propanediol monoacetate	313			
ethyl 9-decanoate		515	274	305
2-phenyl ethyl acetate		46	42	51
diethyl-2-hydroxypentanedioate	337 ^b			
ethyl-2-hydroxy-3-phenyl propanoate	358 ^b			
monoethyl succinate	21337			
ethyl 4-hydroxy-glutarate	587			
isopropyl dodecanoate		11		
ethyl pyroglutamate	77			
ethyl dodecanoate		121	23	79
isoamyl decanoate		6		
monoisoamyl succinate	646 ^b			
isoamyl lactate	87 ^a			
hexyl butanoate		8		
ethyl phenyl lactate	352			
ethyl 3-hydroxy-3-methyl butyrate	11 ^a			
ethyl 2-hydroxy-3-methyl-butyrate	60 ^a			
ethyl pyruvate	22 ^a			
ethyl 4-acetyloxy-butyrate	1020 ^a			
ethyl citrate	80 ^a			
4-ethyl-phenyl acetate	14 ^a			
ethyl 3-hydroxy-hexanoate	264 ^a			
ethyl n-propyl succinate	49 ^a			

ethyl isoamyl succinate		87	45	53
ethyl p-hydroxycinnamate			841	
ethyl pentadecanoate		10		
ethyl hexadecanoate		17		17
Total average	47791	9308	8030	4843
Sulfur compounds				
2-methyl-dihydro-3(2H)-thiophenone + 2-methylthioethanol	46			
3-methylthio-1-propanol	396			
dimethylsulphone	25		6	
3-methylthio-propanoic acid	43			
methyl thioacetate	5			
3-ethylthio-1-propanol	65 ^a			
Total average	580		6	
Nitrogen compounds				
N-3-methylbutyl acetamide	136			
2-phenylethyl acetamide	72			
N-acetyl glycine	79 ^a			
N,N-diethylbencylamine	29 ^a			
ethyl N-acetyl methionine	15 ^a			
Total average	331			
Furfural and furanic derivatives				
furfural	1623	26	231	84
5-hydroxy-methylfurfural	290			
2-acetylfuran	63			
5-methyl-furfural	305 ^a	18	25	32
furfuryl alcohol	194			
isobenzofuranone	42			
ethyl-2-furoate	5 ^b			
4,6-dimethyl-2H-pyran-2-one	5			
2-hydroxy-4-pyranone	119			
Total average	3772	44	256	116
Lactones				
g-butyrolactone	2334			
4-ethoxy-g-butyrolactone	15			
whiskey lactone (Z)	181		10	
4-carbethoxy-g-butyrolactone	481			
	LLE	HS-SPME		
Compound	($\mu\text{g L}^{-1}$)	($\mu\text{g L}^{-1}$)		
	PDMS			PA
Lactones Cont.				
g-crotonolactone	16 ^a			
whiskey lactone (E)	60 ^a		12	
d-octalactone	11 ^a			
mevalonic lactone	62 ^a			
g-decanolactone	31 ^a			
g-5-hydroxy-hexalactone	210 ^a			
Total average	3462		22	
Volatile phenols				
siringaldehyde	293 ^b			
ethyl syringoate	106			
syringone acetate	123 ^b			
2-methoxyphenol	21			

4-vinylguaiacol	13			
2,6-dimethoxyphenol	39			
4-allyl-2,6-dimethoxyphenol	29 ^b			
guaiacyl ethanol	73			
guaiacyl propanol	55			
3,4,5-trimethoxyphenol	77			
4-methyl guaiacol	14 ^b			
guaiacyl ketone	15 ^b			
ethyl guaiacyl propanoate	9			
4-methoxyacetophenone	320 ^a			
phenylethyl benzoate	111 ^a			
ethyl 4-hydroxy-benzoate	75 ^a			
guaiacol	11 ^a			
Total average	1384			
Shikimic acid derivatives				
vanillin	118			
ethyl vanillate	83			
acetovanillone	62			
propiovanillone	23			
vanillyl methyl ketone	57			
methyl vanillate	2 ^a			
Total average	344			
Others				
E-4-hydroxymethyl-2-methyl-1,3-dioxolane	41			
4CALA precursor	55 ^b			
Total average	96			

^(a) compound only detected for Maipo valley wines; ^(b) compound only detected for Colchagua valley wines

Analysis of Carménère wines by Head Space Solid Phase MicroExtraction (HS-SPME)

More than 60 volatile compounds could be extracted with HS-SPME technique (Table 1). Because a single fiber cannot extract all the volatiles and their different extraction selectivity, three types of fibers, coated with polymers with increasing polarity, were employed for this purpose: polydimethylsiloxane (PDMS, apolar), divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, medium polar) and polyacrylate (PA, polar). The compounds were thermally desorbed and further identified by GC/MS.

With this extraction technique also the main groups of chemical compounds found were esters and alcohols, although in different proportions according to the polarity of the fiber used. Thus, for the PDMS and DVB/CAR/PDMS fiber 78% and 76% of esters, respectively, and 17% of alcohols were extracted. On the other hand, the PA fiber was slightly more selective to alcohols with 38% of alcohols and 59% of esters.

Interestingly, some volatiles that could not be detected by LLE were revealed by HS-SPME. The apolar and the mixed polymer coating fibers were able to extract three monoterpenes: linalool, citronellol and geranyl acetone; a sesquiterpene, nerolidol; and a norisoprenoid, b-damascenone. The latter was a particularly interesting compound, because it has been described as an important odorant in red wines with notes of baked apples, marmalade and honey^{51,52}.

The PA fiber was more selective to alcohols like isoamyl alcohol and 2-phenyl ethanol. However, DVB/CAR/PDMS fiber extracted methionol (3-methylthio-1-propanol), whiskey lactones (coconut) and ethyl p-hydroxycinnamate (flowery); and the PDMS fiber allowed to extract a higher number of carbonylic compounds. Canuti et al³¹ recently reported that the use of PDMS fiber resulted in greater extraction of norisoprenoids (e.g., damascenone) and terpenes (e.g., linalool), as well as alcohols and the more polar aldehydes, in agreement with our results.

Minor varietal compounds

Some minor varietal compounds, in particular methoxypyrazines and

thiols, were also detected. Although these compounds were present at very low concentration, their low odor perception threshold, at the ppt level, generally makes them responsible for a significant impact in the total aroma of wines and other food matrices. 2-isobutyl-3-methoxypyrazine (green bell pepper notes), was identified in the wines at an average concentration of 3,6 ng L⁻¹. It is worthy to mention that the IBMP concentration in the Carménère wines studied here was much lower than the ones reported by Belancic and Agosin¹⁶, for 2003 Carménère wines, suggesting that, currently, a better management of this herbaceous character is conducted. 2-isopropyl-3-methoxypyrazine was not detected.

Four thiols were detected and quantitated, too (Table 2). These compounds give to wines, fruits and other diverse sensory notes and some, like 4-mercapto-4-methylpentan-2-one or 3-mercaptohexan-1-ol for example, have been classified as high impact odorant compounds in white wines like Sauvignon Blanc⁵³. Although 4MMP was detected in Carménère wines, its concentration was very low and it could not be quantitated. 2-furanmethanethiol has been reported for wines aged in oak barrels and in champagne, contributing with toasted aroma. It was found also in red wines with concentrations between 25 and 140 ng L⁻¹³⁴. Moreover, benzenemethanethiol was identified in boxwood as well as in red and white wines at concentrations as high as 30-100 fold higher than their perception threshold⁵³. These compounds were found in the young Carménère wines from Colchagua and Maipo valleys in concentrations lower than the reported range, but their contribution to the total aroma could not be discarded, because concentrations are greater than their olfactory threshold. 3-mercaptohexyl acetate and 3-mercaptohexan-1-ol have also been identified in Sauvignon Blanc, Riesling, Gewürztraminer, Cabernet Sauvignon and Merlot variety⁵⁴. 3MH was reported to have a definitive impact on the fruity aroma of Bordeaux rose wines⁵⁵. Both compounds were also found here with levels over their odor detection threshold (ODT).

Table 2. Minor varietal compounds in Carménère wines.

Compound	Average Conc. (ng L ⁻¹)	Literature aroma descriptor
Pyrazines		
IBMP	3,6	bell pepper, vegetative, gas
Thiols		
2FM	10,1	roasted coffee
BM	14,1	smoky, metallic
4MMP	nq	box tree, guava aroma, cat urine, passion fruit
3MH	666,8	fruity, animal, grape, box tree, broom, grapefruit
3MHA	373,4	box tree, passion fruit, broom
<i>Not quantified compound (nq)</i>		

Estimation of high impact olfactory compounds

An estimation of the potential active odorant compounds of young Carménère wines was carried out using the odour activity value²⁰. The OAV value of any volatile is calculated as the ratio between the measured concentration of a substance in the wine and its odour perception threshold, reported in the literature. This scale allows estimating the relative impact of each compound to the wine aroma. Compounds with OAV>1 are considered to contribute individually to the wine aroma, although it has been suggested that it is also necessary to consider the sensory contribution of those substances with OAV>0.2, because of the additive effect of similar compounds with similar structure or odour^{56,57}.

Table 3. Estimated OAV average values for odorants compounds identified in Young Carménère wines from Colchagua and Maipo valley.

Compound	Aroma descriptor	Odour threshold ^{Ref.} (µg L ⁻¹)	OAV
Carbonyl compounds			
2,3-butanedione	buttery/caramel	100 ^a	7,3
Alcohols			
isoamyl alcohol	fussel alcohol, grass, bitter, harsh	30000 ^b	1,6
2-phenylethanol	rose, flowery	14000 ^c	1,5
Lactones and shikimic acid derivatives			
whiskey lactona (Z)	sweet, coconut	25 ^d	7,2
4-carbomethoxy-g-butyrolactone	red fruits, sherry ^e	400 ^e	1,2
whiskey lactone (E)	sweet, wood, fruit	110 ^d	0,5
vanillin	cake, vanilla	60 ^c	2,0
Acids			
isobutyric acid	acid, fatty	230 ^f	1,4
butyric acid	cheese, rancid	173 ^f	1,3
hexanoic acid	fatty, cheese	420 ^f	2,6
octanoic acid	fatty	2200 ^e	0,8
decanoic acid	rancid, fat	1000 ^f	0,4
phenyl acetic acid	honey, flowery	1000 ^f	0,2
isovaleric acid	blue cheese	250 ^e	4,0
Esters			
ethyl isobutyrate	fruity	15 ^b	1,2
ethyl butyrate	kiwi, acid fruit	20 ^c	6,0
ethyl isovalerate	fruity, anise	3 ^f	2,9
isoamyl acetate	fresh, banana	30 ^c	30,3
ethyl hexanoate	fruity, green apple, anise	14 ^g	19,1
ethyl octanoate	fruity, sweet, soap, anise	5 ^g	99,9
2-phenylethyl acetate	roses	250 ^g	0,6
ethyl -2-methyl-butyrate**	red fruits	18 ^f	0,3
Sulfur and nitrogen compounds			
3-methylthio-1-propanol	potato, soup, meat	1000 ^g	0,4
2-furanmethanethiol*	roasted coffee	0,0004 ^h	25,3
benzenemethanethiol*	smoky, metallic	0,0003 ⁱ	47,4
3-mercaptohexan-1-ol*	fruity, grape, box tree, grapefruit	0,060 ^j	11,1
3-mercaptohexyl acetate*	box tree, passion fruit, broom	0,004 ^j	93,4
2-methoxy-3-isobutyl-pyrazine*	green bell pepper	0,002 ^k	1,8
Terpenes and norisoprenoids			
linalool**	orange flowers	25 ^l	0,3
b-damascenone**	baked apple, tea, flower, peach	0,05 ^g	516,2

(*) compounds quantified using specific methods; (**) compounds detected with SPME (PDMS) extraction technique

(Ref) Odor threshold reference: a⁶⁰ (Santos 2009); b⁴¹ (Moreno 2005); c⁶¹ (Culleré 2008); d⁴⁴ (Fernández de Simón 2003); e⁵⁶ (Rocha 2004); f⁵⁷ (Vilanova 2009); g³⁵ (Yongsheng Tao 2008); h³⁴ (Tominaga 2006); i⁵³ (Tominaga 2003); j⁵⁴ (Tominaga 2006); k¹⁶ (Belancic 2007) and l²⁰ (Zalacain 2007).

Table 3 contains the compounds present in the Carménère wines with significant OAV values. b-damascenone could be considered as the most powerful odorant (OAV=516) in agreement with the information reported for other red wines⁵¹ and some white wines from Galicia⁵⁷. Ethyl octanoate and 3-mercaptohexyl acetate showed also very high values, around 100 OAV, in average. Several other compounds also exhibited OAV>1 like the esters ethyl hexanoate, ethyl butyrate, ethyl isovalerate and isoamyl acetate. Esters contribute favourably to wine aroma with fruity characteristics. Among the varietal compounds the thiols benzenemethanethiol (smoky), 2-furanmethanethiol (toasty) and 3-mercaptohexan-1-ol (grapefruit, tropical fruits) also contribute to the Carménère aroma. Fatty acids: C4-C6 (sour, rancid, fatty, cheesy notes); the alcohols: 2-phenylethanol (roses scent) and isoamyl alcohol (fusel alcohol); diacetyl (buttery/caramel); and the lactones: whisky lactone (woody, coconut) and the 4-carbetoxy-g-butyrolactone (red berries, sherry nuances) were in concentrations greater than their ODT. However, the contribution to the total aroma of substances with near-unity OAVs cannot be ignored, because they could enhance some existing notes by synergy with other compounds.

CONCLUSIONS

The Carmenerre wines studied here belong to two premium wineries, Casa Silva and Perez Cruz, located at two different Chilean viticultural regions (Colchagua and Maipo valleys). Liquid-liquid extraction and headspace solid phase microextraction methods showed to be complementary in the characterization of the aroma profile of Carménère wines. Except for some differences that could be associated with the terroir or prefermentation and vinification practices, the two Carménère wines had similar features: the varietal compounds 2-isobutyl-3-methoxypyrazine, benzenemethanethiol, 2-furanmethanethiol, 3-mercaptohexan-1-ol, 3-mercaptohexyl acetate and the norisoprenoid b-damascenone constitute potential high impact odorants of the aroma of Carmenerre wines. Isobutylmethoxypyrazine and several thiols, present in trace levels concentration, were also found to be significant contributors. On the other hand, fermentative compounds, such as esters (isoamyl acetate, ethyl octanoate and ethyl hexanoate), alcohols (isoamyl alcohol, 2-phenyl ethanol) and fatty acids (butyric, hexanoic and octanoic acids), as well as wood-derived volatiles, like lactones (whiskey and 4-carbetoxy-g-butyro lactone), could also be involved in the aroma of these wines. Nevertheless, although the contribution to overall aroma of varietal substances, such as 3-oxo-a-ionol, ethyl hydroxybutanoate and the terpenes linalool, citronellol and nerolidol could not be demonstrated, their contribution to the whole aroma, alone or in combination with other compounds could not be totally discarded.

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