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Analysis of Sparkling Wine Lees Surface Volatiles by Optimized Headspace Solid-Phase Microextraction

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During sparkling wine aging, a narrow interaction is established between wine and lees of second fermentation, which remain in contact during long periods. In order to contribute to the knowledge on this interaction, volatile compounds retained by lees were described in this study. With this aim, solid phase microextraction (SPME) conditions were optimized in order to increase the sensibility for the analysis of volatiles sorbed by lees. This allowed proving the capacity of sparkling wine lees of second fermentation to retain several volatile compounds related with wine aroma: esters, aldehydes, norisoprenoids and terpenes known for their positive flavor impact were found in lees headspace. Most of them were previously described in "Cava" sparkling wine, while some compounds, such as the tentatively identified trimethyl tetrahydronaphthalenes, were not previously identified in yeasts or wine.

KEYWORDS: Lees; sparkling wine; aroma; sorption; solid phase microextraction

INTRODUCTION

Cava sparkling wines are elaborated using the traditional practice of "sur lie" aging that includes a long time of contact between lees and wine (minimum nine months). During this period, different compounds such as lipids, carbohydrates, amino acids, peptides and volatiles could be released in a process known as autolysis (*1*). This process has a very important role in sparkling wines' "bouquet" conformation (*2*). Finally, when the over-lees is finished, the lees are removed from the bottle becoming a residue.

Lees are composed mainly by yeast cells (*3*), and it is the outer part of yeasts, which is in constant contact with wine. This cell wall makes up between 25 and 50% of cell volume (*4*), and it is structured in an inner network of ramified glucans and outer layer of mannoproteins (*5*). These compounds confer to cells physicochemical properties that enable yeasts and lees to interact with other compounds. Yeast cells have shown differences in their wall composition depending on the species and strains. Therefore, this diversity in molecules could affect the interaction with wine (*6*). The sorption capacity of yeasts and lees surface toward several organic substances has been proved, such as in the case of wine polyphenols (*7*). In most cases the interactions between yeast and organic compounds were exploited for the natural removal of undesirable compounds from wine, such as toxins, pesticides, antifoaming and volatile compounds (*8*). The study of yeasts' sorption potential for the removal of undesirable volatiles in wine was principally focused on volatile phenols (*9*−*11*). Moreover, the sorptive properties of this biological material were proved by adding yeast cell walls to a model wine containing esters, higher alcohols and β-ionone and obtaining the increase of their retention in solution (*12*). In particular, proteins were the cell wall component claimed to bind volatile compounds (*9*−*11*).

During sparkling wine aging, a narrow interaction is established between wine and lees of second fermentation, which remain in contact during long periods. The exchanges of volatile compounds between wine and lees are thought to be a main aspect in the development of cava sparkling wine *bouquet* during aging (*1*). A decrease of volatile compounds after lees contact was reported in white wines, demonstrating the importance of lees sorption phenomena on wine aroma (*13*). Moreover, the capacity of lees to retain aromatic substances could be of great interest for the aroma industry, for the possible use of this byproduct once the biological aging of wine has finished. At present, wine lees are already used for the recovery of tartaric acid (*14*).

The ability of yeast lees to modify the volatile composition of wines has been indirectly evaluated by analyzing the volatiles of wines after the exposure to lees (*15*−*17*). To our knowledge, the study of the volatiles directly retained by the lees surface has never been performed. Only a few studies detecting volatile compounds in commercial yeast extracts or pastes are available in literature (*18, 19*), but in these cases, the volatile compounds are mainly attributed to thermal generation due to industrial production processes. As well, Kotseridis et al. (*20*) reported the volatiles of some commercial dried yeast before fermentation.

The aim of the present work was to describe the volatiles retained by lees during second fermentation of sparkling wines. With this purpose, an optimized headspace solid phase mi-

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croextraction method coupled to gas chromatography and mass spectrometric detection (SPME−GC/MS) was developed and applied to a heterogeneous group of sparkling wines lees in order to obtain a wide qualitative profile of lees' surface volatiles.

MATERIAL AND METHODS

Sample Preparation. The experimental design for the optimization of the analytical method was carried out with lees of the same batch of sparkling wine bottles.

Once the extraction conditions were established, the lees of six sparkling wines pertaining to the categories Cava, Cava Reserva and Cava Gran Reserva were analyzed. Two different bottles were analyzed for each type of cava (total 6 × 2, 750 mL bottles).

Prior to the SPME analysis, the lees were prepared as follows: the content of 1 bottle was centrifuged for 15 min at 1410*g* and 4 °C (Rotina 48CR); the pellet was resuspended in 10 mL of the same wine, then filtered through 0.45 μm pore size cellulose filter (Sartorius Stedim Biotech, Goettingen, Germany) and finally washed with 2 mL of NaCl 0.9% (Panreac, Barcelona, Spain). Between 300 and 500 mg of lees were obtained after this process.

SPME Conditions. The SPME fiber used was divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 50/30 μm, 2 cm long, from Supelco (Bellefonte, PA).

For the analysis, 25 mg of lees was placed into a 10 mL vial and suspended in different volumes of NaCl solution. The vial was then fitted with a silicone septum, and the lees were maintained in suspension by means of magnetic stirring (700 rpm). After 10 min of sample conditioning, the fiber was exposed to the sample headspace during 40 min.

Multilevel Factorial Experimental Design. The variables tested were as follows: sample dilution with NaCl solution 0.9% (1 or 4 mL), extraction temperature (40 or 50 °C) and pH of NaCl solution (pH 4 or pH 7). The solution at pH 4 was achieved by adjusting the NaCl solution 0.9% with HCl 0.1 N (Panreac, Barcelona, Spain) before suspending the lees, while a 10 mM phosphate buffer in NaCl 0.9% was used to suspend the lees at pH 7. The pH of each suspension was checked after the extraction and confirmed to maintain at values of 4 and 7, according to the solution used.

The optimized experimental design involved 16 experiments. The best extraction conditions were as follows: volume of suspension 1 mL at pH 7, and extraction temperature 50 °C.

GC−MS Analysis. Identification of compounds was performed by gas chromatography coupled to quadrupolar mass selective spectrometry using an Agilent 5973 Network detector (Agilent Technologies, Palo Alto, CA). Analytes were separated on a Supelcowax-10 (Supelco) 30 m × 0.25 mm i.d., 0.25 μm film thickness and on a Equity DB-1 Fused Silica Column (Supelco) 60m × 0.25 mm i.d. 0.25 μm film thickness. Column temperature was held at 60 °C for 3 min, increased to 75 at 4 °C/min, then to 260 at 8 °C/min, holding 5 min. The injector temperature was 260 °C and the time of desorption of the fiber into the injection port was fixed at 5 min. Helium was the carrier gas, at a linear velocity of 38 cm/s. The temperature of the ion source was 175 °C and the transfer line, 280 °C. Electron impact mass spectra were recorded at 70 eV ionization energy, 2 scan/s. GC−MS analysis in the complete scanning mode (SCAN), in the 40−300 u mass range, was performed to allow the identification of compounds in lees samples.

Determination of the Linear Dynamic Range. In order to establish an interval of sample weight allowing the detection of the maximum number of compounds, but avoiding the saturation of the fiber, different amounts of lees were analyzed at the optimized extraction conditions.

Characterization of Volatiles in Lees. Compounds were identified by comparison of their mass spectra and retention times with those of standard compounds, or by comparison of the mass spectrum with those of the mass spectra library Wiley sixth. Reference compounds used for the identification of lees volatiles were purchased by Sigma-Aldrich (S. Louis, MO). Kovats' retention indices were determined with reference to a homologous series of linear alkanes and compared with those available in the literature for two capillary columns with different polarity.

Statistical Analysis. The statistical data were obtained using Statgraphics Plus 5.1. The significance of the factors studied in the experimental design and the optimum values for each factor were established by means of ANOVA and regression model analyses, respectively. Simple linear regression was used to evaluate the dependence between lees weight and chromatographic response. Results were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Effect of Extraction Conditions. *Preliminary Assays.* DVB/CAR/PDMS was the fiber selected for the study in view of its capacity to extract a broad variety of analytes. Furthermore, the suitability of this fiber for wine volatile profile analysis, compared with other commercial coatings, has been previously reported (*21*−*23*).

A preliminary comparison of the SPME uptakes achieved by analyzing filtered lees and lees in suspension was performed. The chromatographic response of 25 mg of lees after filtration was compared with that obtained analyzing 25 mg of the same lees resuspended in 4 mL of NaCl 0.9%, and maintained under magnetic stirring (700 rpm). An increase of 54% in the total chromatographic area was observed by analyzing lees in suspension (data not shown). The higher effectiveness of this extraction mode could be due to higher volatility of compounds in aqueous solution, and justified by a higher exposition of the lees surface compared to direct extraction of wet lees, which tend to agglomerate. For the same reason, this extraction mode should improve the repeatability of the extraction.

A suitable extraction time was also previously established by analyzing 25 mg of lees suspended in 1 mL of NaCl 0.9%, at 50 °C and under magnetic stirring, during 30, 40 and 60 min. An enhancement of 19% in the total chromatographic area was observed by increasing the extraction time from 30 to 40 min, while a further increase of the extraction time to 60 min only resulted in a 1% of increase of the response (data not shown). On this basis, the extraction time was fixed in 40 min.

Experimental Design Evaluation. The influence of each variable on the chromatographic response of major compounds was evaluated by multifactor ANOVA analysis of the experimental design's results. Moreover, the optimal extraction conditions were statistically established on the basis of final regression models from the factorial design results (**Table 1**). The factors studied were extraction temperature (*T*), sample volume (Vol) and pH of the aqueous phase. Extreme conditions of pH, temperature or ionic strength were avoided in order to preserve the structure of the yeast cells.

The number and state of cells were microscopically analyzed before and after the extraction. Neither significative reduction in cell number nor important changes in cell morphology were observed, leading us to suppose that in all the cases the integrity of lees was preserved. Moreover, quantitative rather than qualitative differences were found between the volatile profiles obtained in the distinct conditions assayed, suggesting that none of these conditions induced the generation of specific artifacts.

For most of the compounds evaluated, the best results were obtained at the extraction temperature of 50 °C and by diluting lees in 1 mL of NaCl solution (**Table 1**), likely because a smaller solvent volume involved a higher concentration of volatiles in solution (*24*) and a higher temperature enhanced their volatilization. Finally, the compounds whose uptakes were significantly influenced by pH showed better extraction results when SPME extraction was carried out at pH 7, excepting decanoic acid, β-farnesene, diethyl succinate and geranyl acetone. Furthermore, at this pH value a positive significant interaction with temperature and volume was observed for many compounds, which

Table 1. Results of the Factorial Experimental Design for the Extraction of Volatiles in Sparkling Wine Lees[a]

compound	factor			significant interaction[e]		
	T (A)[b]	Vol (B)[c]	pH (C)[d]	AB	AC	BC
hexanal	50	1				*
heptanal	50	1		*		
limonene	50	4		*	*	*
ethyl hexanoate	50					
octanal	50	1			*	*
6-methyl-5-hepten 2-one	50					*
1,1,6-trimethyl-1,2,3,4-tetrahydronaphthalene	50	1				
methyl octanoate						
nonanal	50	1	7	*		
ethyl octanoate	50					
isoamyl hexanoate	50	1			*	
2-ethylhexanol		1				
decanal	50					
propyl octanoate	50	1			*	
vitispirane isomer	50	1				
vitispirane isomer	50	1				
ethyl nonanoate	50	1	7		*	
methyl propyl octanoate	50	1				
trimethyl tetrahydronaphthalene isomer	50	1	7	*		*
methyl decanoate	50	1	7			
ethyl decanoate	50	1	7			
isoamyl octanoate	50	1	7		*	
trans-β-farnesene	50	1	4	*		*
diethyl succinate	50	1	4		*	*
ethyl-9-decenoate	50	1	7			
propyl decanoate	50	1	7			
α-muurolene	50	1			*	
6-(1,1,dimethylethyl)-1,2,3,4, tetrahydronaphthalene		1				*
1,1,6-trimethyl-1,2-dihydronaphthalene (TDN)	50	1				
decanol	50	1				
cis-α-bisabolene	50	1				
hexyl octanoate	50	1				
α-farnesene	50	1			*	
1-(2,4,6-trimenthylphenyl)buta-1,3 diene (TPB)	50	1	7	*		
ethyl laurate	50	1				
geranyl acetone	50		4			*
isoamyl decanoate	50	1				
ethyl-9-hexadecenoate	50	1	7	*	*	*
sesquiterpene	50	1				
nerolidol	50	1	7		*	*
ethyl myristate		1				
6-methoxy-1-acetonaphthone	50	1				
α-bisabolol	50	1	7			*
sesquiterpene	50					
decanoic acid			4			

[a] The significance of each factor and interaction was tested by ANOVA. The optimal values, established by final regression models from the factorial design results, are reported only for significant factors. [b] Temperature. [c] Volume. [d] pH. [e] $p < 0.05$. *Significant interaction ($p < 0.05$) between two factors.

determined an enhancement of their extraction. The effect of pH on the extraction of volatile compounds could be due to the modification of the charge of macromolecules like mannoproteins, which were proposed to be mainly responsible for the sorption of some compounds in yeast's cell wall (*10*).

On the basis of the above-mentioned results, the following conditions were considered as the most suitable for the analysis of volatiles retained on lees surface: volume of suspension 1 mL, extraction temperature 50 °C and pH 7 (10 mM phosphate buffer in NaCl 0.9%).

Effect of Sample Size on SPME Analysis. The SPME coating possesses a limited number of sorption sites, and an excess of analytes could cause the saturation of the fiber (*24*). This effect would make difficult the appreciation of quantitative differences in the volatile profiles of distinct samples. To ensure performing the analysis below the saturation of the fiber and to establish an amount of sample sufficient to detect minor volatiles, different weights of lees were analyzed at the optimized extraction conditions and the responses given were evaluated. **Figure 1** illustrates the chromatographic response of four representative compounds belonging to different chemical families, as a function of sample weight. For all the volatiles a significant ($p < 0.05$) linear correlation was observed in the range of 4−37 mg of lees between the sample weight and the chromatographic response ($r > 0.90$). The amount of lees used in the experiments was 25 mg, which was situated within the linear section and allowed the detection of some minority compounds.

Volatiles Detected in Sparkling Wine Lees. *Major Compounds.* Fifty-seven compounds were identified or tentatively identified by applying the optimized SPME−GC/MS method for the analysis of lees from twelve samples of sparkling wines pertaining to six distinct commercial categories. Major compounds of samples' headspace are reported in **Table 2**, together with their identification parameters. Although the quantification of volatiles in lees samples was not considered in the present study, the relative abundance of volatiles (expresses as area counts) was reported in order to assess their relative proportions in the volatile profile of lees. Several esters, aldehydes, norisoprenoids, and terpenes constituted the major compounds

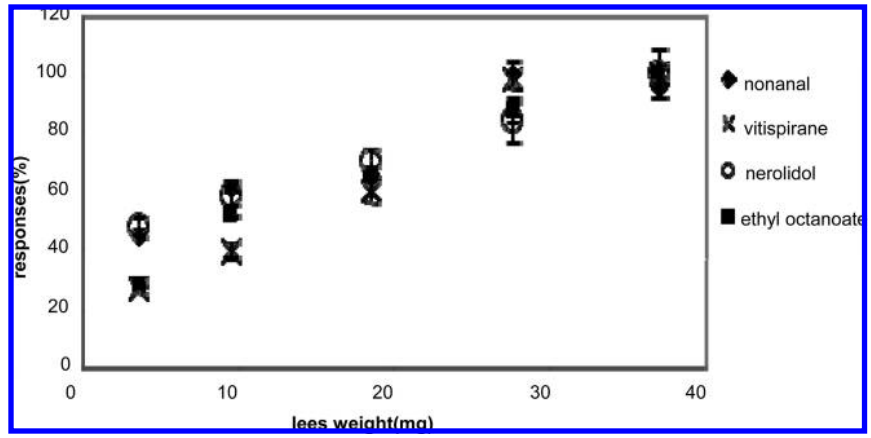

Figure 1. Normalized response of nonanal, ethyl octanoate, vitispirane, and nerolidol obtained from different weights of lees. The results are expressed as percent of normalized chromatographic areas.

Table 2. Major Volatile Compounds in the Headspace of Lees from Sparkling Wine Samples ($n = 12$) of Different Commercial Categories

			Carbowax		DB-1		abundance[d]		
code	compound	ions[a]	KI[b]	KI lit.[c]	KI	KI lit.	min	max	ID[e]
1	hexanal	44, 56, 100	1064	1084 (*25*)			nd[f]	7.6E06	S,[g] MS,[h] RI[i]
3	limonene	68, 93, 136	1176	1178 (*25*)	1030	1025 (*26*)	nd	3.0E06	S, MS, RI
4	ethyl hexanoate	88, 99, 142	1219	1220 (*25*)	983	980 (*26*)	2.5E06	6.5E07	S, MS, RI
5	octanal	41, 57, 128	1276	1280 (*25*)			nd	2.4E06	S, MS, RI
6	6-methyl-5-hepten 2-one	55, 69, 108, 126	1324		968	969 (*26*)	nd	1.4E06	S, MS, RI
9	methyl octanoate	74, 87, 158	1374	138 3(*25*)			nd	3.8E06	S, MS, RI
10	nonanal	57, 70, 142	1379	1385 (*27*)	1087	1103 (*26*)	2.5E06	1.1E07	S, MS, RI
11	ethyl octanoate	88, 101, 172	1427	1436 (*25*)	1185	1173 (*26*)	1.2E09	4.6E09	S, MS, RI
12	isoamyl hexanoate	70, 99, 186	1443	1451 (*27*)	1232	1233 (*27*)	nd	3.0E07	MS, RI
14	2-ethylhexanol	57, 43, 127	1469				nd	2.6E06	MS
15	decanal	57, 70, 128	1482	1484 (*28*)	1193	1184 (*26*)	2.3E06	1.1E07	S, MS, RI
16	propyl octanoate	141, 27, 186	1499	1508 (*28*)	1268	1270 (*26*)	3.1E06	1.3E07	MS, RI
17	vitispirane isomer	93, 121, 192	1510	1508 (*27*)	1280	1270 (*27*)	1.0E07	8.0E07	MS, RI
18	vitispirane isomer	93, 121, 192	1513	1511 (*27*)			1.6E07	7.7E07	MS, RI
19	ethyl nonanoate	88, 101, 186	1516	1528 (*28*)	1271	1279 (*26*)	3.6E06	8.8E06	S, MS, RI
21	methyl propyl octanoate	127, 157, 200	1534				3.4E06	1.4E07	MS
23	methyldecanoate	74, 87, 186	1576	1590 (*25*)			nd	3.8E06	S, MS, RI
24	2-undecanone	58, 43, 170	1580	1606 (*25*)			2.5E06	1.1E07	S, MS, RI
26	ethyl decanoate	88, 101, 200	1629	1636 (*25*)	1307	1308 (*26*)	2.8E09	5.3E09	S, MS, RI
27	isoamyl octanoate	70, 43, 199	1645	1647 (*25*)	1385	1377 (*26*)	1.0E08	2.2E08	S, MS, RI
29	diethyl succinate	101, 129, 174	1656	1690 (*29*)			nd	2.2E07	S, MS, RI
30	ethyl-9-decenoate	88, 55, 198	1675	1691 (*27*)			1.9E08	1.0E09	MS, RI
31	ethyl *cis*-4-decenoate	88, 55, 198	1684				nd	9.4E06	MS
33	propyldecanoate	173, 155, 214	1702		1472	1473 (*26*)	nd	8.0E06	MS, RI
36	1,1,6-trimethyl-1,2-dihydronaphthalene (TDN)	157, 142, 172	1731	1731 (*27*)	1358	1336 (*27*)	1.0E08	5.6E08	MS, RI
37	decanol	55, 70, 112	1737	1763 (*28*)			nd	3.6E06	S, MS, RI
39	hexyl octanoate	145, 84, 228	1788	1796 (*28*)			2.5E06	5.0E06	S, MS, RI
40	*trans*-β-damascenone	69, 121, 207	1800	1820 (*20*)			5.9E05	3.6E06	MS, RI
42	1-(2,4,6-trimenthylphenyl)buta-1,3 -diene (TPB)	157, 142, 172	1811	1826 (*28*)			1.8E06	9.8E06	MS, RI
43	ethyl laurate	88, 101, 228	1822	1851 (*29*)			1.2E08	3.7E08	S, MS, RI
44	geranylacetone	43, 69, 194	1833	1840 (*25*)			nd	1.9E06	S, MS, RI
45	isoamyldecanoate	70, 155, 242	1840				2.9E07	9.3E07	MS, RI
46	ethyl-9-hexadecenoate	55, 69, 282	1863	1853 (*27*)	1633	1633 (*26*)	2.7E06	1.5E07	MS, RI
51	ethylmyristate	88, 101, 256	2020				4.5E06	2.5E07	S, MS, RI
54	ethylpalmitate	88, 101, 284	2213	2229 (*28*)			9.7E06	5.7E07	S, MS, RI
55	ethyl-9-hexadecenoate	55, 69, 282	2227				3.1E07	1.9E08	MS
57	decanoic acid	73, 60, 172	2240	2307 (*27*)			6.9E06	1.0E08	S, MS, RI

[a] Principal mass spectra fragments. [b] Kovats' retention index. [c] Kovats' retention index reported in literature. [d] Interval of abundance expressed as area counts, obtained analyzing lees samples from different sparkling wines. [e] Identification method. [f] nd: not detected. [g] S: Identified by comparison with standard compounds. [h] MS: Tentatively identified by mass spectra (comparison with NIST and Wiley libraries). [i] RI: Tentatively identified by retention index.

in the headspace of sparkling wine lees. As expected, most of the volatiles retained by the lees surface were previously reported in cava (*22*, *27*). The long periods of contact between lees and wine during the biological aging promoted their interaction and permitted the establishment of partition equilibriums.

Esters have a great importance in the chromatographic profile of both wines (*27*, *29*, *30*) and lees (**Table 2**). The cell surface could retain these compounds once the fermentation has finished or adsorb them during wine aging. Vitispiranes, 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), β-damascenone and 1-(2,4,6-trimethylphenyl)buta-1,3-diene (TPB) were the major norisoprenoids identified on lees surface (**Table 2**). Increasing TDN and vitispirane amounts were observed in Cava during the aging in contact with lees (*22*), while TPB is a potent odorant identified in crude glycosidic

Table 3. Minor Volatile Compounds in the Headspace of Lees from Sparkling Wine Samples ($n = 12$) of Different Commercial Categories, Determined by Analyzing Typical Mass Spectra Fragments

			Carbowax		DB-1		abundance[d]		
code	compound	ions[a]	IK[b]	IK lit.[c]	IK	IK lit.	min	max	ID[e]
2	heptanal	70, 44, 114	1169	1174 (*25*)			nd[f]	1.4E05	S,[g] MS,[h] RI[i]
7	1,1,6-trimethyl-1,2,3,4-tetrahydronaphthalene	159, 144, 174	1350	1347 (*46*)			nd	3.0E05	MS, RI
8	trimethyl tetrahydronaphthalene isomer	159, 144, 174	1372				nd	3.0E05	MS
13	trimethyl tetrahydronaphthalene isomer	159, 144, 174	1458				nd	1.1E05	MS
20	linalool	71, 93, 121, 153	1524	1540 (*27*)			nd	7.4E05	S, MS, RI
22	trimethyl tetrahydronaphthalene isomer	159, 144, 174	1539				nd	1.7E06	MS
25	trimethyl tetrahydronaphthalene isomer	159, 129, 174	1607				nd	9.7E05	MS
28	*trans*-β-farnesene	69, 93, 204	1650	1648 (*25*)	1431	1430 (*26*)	nd	2.6E05	S, MS, RI
32	trimethyl dihydronaphthalene (TDN) isomer	157, 142, 172	1697				1.2E05	5.6E05	MS
34	α-muurolene	105, 161, 204	1709	1714 (*25*)			2.7E05	1.0E06	MS, RI
35	6-(1,1-dimethylethyl)-1,2,3,4-tetrahydronaphthalene	173, 155, 188	1714				5.8E05	3.3E06	MS
38	*cis*-α-bisabolene	93, 119, 204	1751	1778 (*28*)			1.3E05	3.8E05	MS, RI
41	α-farnesene	93, 69, 204	1793	1725 (*28*)			9.0E04	3.8E05	S, MS, RI
47	8-isopropyl-2,5-dimethyl tetrahydronaphthalene	187, 159, 202	1874				nd	1.5E05	MS
48	trimethyl dihydronaphthalene (TDN) isomer	157, 142, 172	1986				6.1E05	2.6E06	MS
49	sesquiterpene	121, 133, 204	1990				2.1E05	4.7E05	MS
50	nerolidol	69, 93, 204	2008	2009 (*28*)			2.6E06	1.5E07	MS, RI
52	6-methoxy-1-acetonaphthone	185, 157, 200	2042		1774	1778 (*26*)	3.7E05	9.4E05	MS, RI
53	α-bisabolol	109, 119, 204	2189	2021 (*28*)			nd	4.2E05	MS, RI
56	dehydroaromadendrene	159, 105, 202	2240	2287 (*46*)			4.23E04	1.7E05	MS, RI

[a] Principal mass spectra fragments. [b] Kovats' retention index. [c] Kovats' retention index reported in literature. [d] Interval of abundance expressed as area counts, obtained analyzing lees samples from different sparkling wines. [e] Identification method. [f] nd: not detected. [g] S: identified by comparison with standard compounds; nd: not detected. [h] MS: Tentatively identified by mass spectra (comparison with NIST and Wiley libraries). [i] RI: Tentatively identified by retention index.

extract of grapes and in white wines (*31, 32*) which has not been described yet in cava sparkling wine.

Although higher alcohols, in particular isoamyl alcohol, represent an important part of the volatile profile of cava sparkling wines (*22, 27*), only small amounts of decanol and 2-ethylhexanol were found in the headspace of lees samples. In agreement with previous studies on yeast wall behavior in model wine (*11*) yeast lees seem to possess a scarce capacity to retain alcohols on their surface.

Organic volatile acids are another major group of compounds in wines' volatile profile that were almost absent in lees headspace. The analytical conditions used in the study are in this case a possible cause of the absence of acids in the samples' headspace because of their dissociation at pH 7.

Unexpectedly, sulfur compounds usually related to wine aging in contact with lees and responsible for empyreumatic notes such as "yeast", "bread", "toasted" or even for olfactory defects (*33*−*37*) were not detected among the volatiles present on lees' surface. Various hypotheses can be made to explain this fact. It could be due to their very low concentrations in wines, or to a scarce affinity of lees surface for these compounds, as already observed for alcohols. On the other hand, the capacity of lees to remove sulfurs from wine was demonstrated (*34, 38, 39*), thus indicating a high capacity of lees to retain these compounds. Nevertheless, the mechanism proposed to explain thiols' removal by lees involves their interaction with thiol groups of lees' mannoproteins, leading to the formation of disulfide bridges (*34*). This covalent binding of thiols to the lees surface would avoid their volatilization and their presence in the headspace.

These results seem to confirm that the nature of the volatiles has an important effect on their sorption by lees surface, as previously demonstrated for yeast walls (*11*). The higher hydrophobicity of esters could explain their larger retention by lees compared with the major wine alcohols, which remained almost undetected in the lees headspace. As well, very hydrophobic minor compounds such as norisoprenoids were retained by lees. In particular, TPB has not been detected in sparkling wine but it was detected in lees, leading us to suppose that very low amounts of TPB could be present in sparkling wine and they are largely sorbed on lees surface.

Minor Compounds. In addition to the major compounds above-described, a number of minor compounds were identified or tentatively identified in the headspace of lees samples by means of analysis of characteristic ions (**Table 3**). Some aliphatic ketones such as geranyl acetone, which is related to the degradation of C13 carotenoids, and 6-methyl-5-hepten-2-one were detected. None of them has been described in Cava wines, but their presence was reported in other wines or in some grape variety (*40*−*42*). As well, six compounds with sesquiterpenic structure were tentatively identified among minor compounds (**Table 3**). Sesquiterpenes were reported to be synthesized in grape berries (*43*) and were described in wines as important sensory contributors (*44*). Some compounds with sesquiterpene structure were already detected in sparkling wine samples (*27, 42*). As far as we know, the sesquiterpenoids tentatively identified as bisabolene, bisabolol and dehydroaromadendrene were not previously identified in wine or grape.

Finally, it is worth mentioning the presence on lees surface of a number of norisoprenoid related compounds, to which trimethyl tetrahydronaphthalene and trimethyl dihydronaphthalene structures were tentatively assigned on the basis of their mass fragmentation (**Table 3**). Compounds characterized by fragments *m*/*z* 157, 142 and 172 were tentatively identified as TDN isomers, while those characterized by the presence of mass fragments *m*/*z* 159, 144 and 174 were tentatively identified as trimethyl tetrahydronaphthalene isomers. It is possible to note that the principal fragments of the latter possess two mass units more than fragments of TDN related compounds.

Trimethyl tetrahydronaphthalenes were found in the volatile profile of tomato paste (*45*) and starfruit (*46*), but to the best of our knowledge, none of these compounds has been previously reported neither in yeast derivates nor in wine. A high affinity of lees surface for these volatiles could explain the detection of these compounds not previously identified in sparkling wine.

Nevertheless, further studies are necessary to confirm their identification and their presence in sparkling wine. The characteristic norisoprenoid structure leads us to suppose that they could be substances with a high sensory impact.

In conclusion, the affinity of sparkling wine lees of second fermentation toward several volatile compounds related with wine aroma was demonstrated by an optimized SPME−GC/MS analysis of lees samples from wines of different commercial categories. Esters, aldehydes, norisoprenoids and terpenes known for their positive flavor impact were found in lees headspace. Most of them were previously described in cava sparkling wine, while some compounds, such as trimethyl tetrahydronaphthalenes, were not previously identified in yeasts or wine. These results corroborate the importance of the surface interaction between lees and wine in the development of sparkling wine characteristics during aging.

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