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Volatile profiles of sparkling wines obtained by three extraction methods and gas chromatography–mass spectrometry (GC–MS) analysis

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Abstract

Simultaneous distillation extraction (SDE) and closed-loop stripping analysis (CLSA) show great capacity for organic compound extraction. Here we used these techniques to obtain and characterize a wide range of volatile compounds from aged cava sparkling wine. We also explored the potential application of head space-solid phase micro extraction (HS-SPME) to determine the distinctive volatile compounds of aged cava as this technique is a common extraction method in quality flavour control. For SDE, 50 mL of cava were extracted with pentane/dichloromethane during 4 h in a Likens–Nickerson (LN) extraction apparatus; while for CLSA, 25 mL of cava were stirred during 1 h into a CLSA apparatus with an adsorbent trap of granulated activated carbon. HS-SPME was performed at 35 °C using 2 ml of sample for 30 min. The 20-mm SPME fibber was coated with a 50/30-μm layer of divinylbenzene–carboxen–polydimethylsiloxane (DVB–CAR–PDMS). All the extraction methods were followed by gas chromatography–mass spectrometry (GC–MS) analysis. SDE and CLSA allowed the identification of 84 volatile compounds. Almost 40% of the volatiles from these two techniques were obtained by HS-SPME. Moreover, here we provide the first description of several tentatively identified compounds such as lilial, octanal, 2-octanone, isopropyl disulfide, methylthiophen-3-one, α-amyl-cinnamaldehyde, ethyl 2-furancarboxylate, 2-acetylfuran, and 5-methylfurfural in cava.

Keywords: Volatile compounds; HS-SPME; CLSA; SDE; Sparkling wine; GC–MS

1. Introduction

The volatile composition of wines is complex because several organic structures contribute to flavour (Aznar, López, Cacho, & Ferreira, 2001; Câmpeanu, Burcea, Doneanu, Nămolosanu, & Visan, 1998; Castro, Natera, Benitez, & Barroso, 2004; Genovese, Dimaggio, Lisanti, Piombino, & Moio, 2005; Karásek et al., 2003; Ortega, López, Cacho, & Ferreira, 2001; Salinas, Alonso, & Esteban-Infantes, 1994; Sánchez-Palomo, Pérez-Coello, Díaz-Maroto, González-Viñas, & Cabezuedo, 2006; Schneider, Baumes, Bayonove, & Razungles, 1998; Zea, Moyano, Moreno, Cortes, & Medina, 2001). The amounts of volatile compounds in wine range from nanograms to micrograms; moreover, these compounds have distinct physicochemical properties regarding, for example, polarity, volatility, and odour impact (Aznar et al., 2001; López, Ferreira, Hernández, & Cacho, 1999) as a result of the functional groups (alcohol, aldehyde, acid, etc.) present in the molecules. Cava is a quality sparkling wine (Certified Brand of Origin) elaborated by the traditional method that consists of a second fermentation followed by biological ageing in contact with lees in

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anaerobic conditions for at least 9 months (Council Regulation (EC), 1493/1999). Volatile compounds in wine have three origins: from the grape (pre-fermentative aroma); from the yeast during the first or second fermentation (fermentative aroma); or from ageing during settling (post-fermentative aroma). This special ageing of cava gives this wine a more complex volatile profile since autolytic and enzymatic reactions may take place. It is thought that the base wine, the autolysis of lees of the second fermentation and the ageing time in contact with these lees are the most important factors that affect the sensory quality of sparkling wines. Aroma is also of paramount importance for producers of high quality sparkling wines such as *champagne* (Vannier, Brun, & Feinberg, 1999). In this regard, the flavour of cava is empirically described by cava makers with yeasty and toasty notes. Although several volatiles, like 1,2-dihydro-1,1,6-trimethylnaphthalene (TDN), vitispiranes isomers and diethyl succinate, have been used as markers of ageing (Riu-Aumatell, Bosch-Fusté, López-Tamames, & Buxaderas, 2006), distinctive compounds involved in the flavour of aged cava have not been characterized to date.

Here we applied a simultaneous distillation extraction method (SDE) and closed-loop stripping analysis (CLSA), both with high uptake techniques, to study a wide volatile profile of aged sparkling wine. SDE (Likens–Nickerson technique) combines the advantages of liquid–liquid and steam distillation extraction, and has been used to isolate volatile organic compounds from spices (Díaz-Maroto, Pérez-Coello, & Cabezudo, 2002) and wine (Blanch, Reglero, & Herraiz, 1996), with very high recovery rates. CLSA has been used to extract pollutants at very low concentrations from water samples (Malleret, Bruchet, & Hennion, 2001). Recently CLSA has been proposed as an effective method for isolating wine aroma compounds (Eggers, Kenefick, Richardson, Wigglesworth, & Girad, 2003). However, neither SDE nor CLSA have been applied to obtain a volatile profile from cava sparkling wines.

Head space-solid phase micro extraction (HS-SPME) is a simple, rapid, solvent-free and inexpensive method for extracting volatile and semi-volatile compounds. It is based on equilibrium between the analyte in vapour phase and silica fibre coated with an adsorbent polar or apolar polymer. A wide range of commercial fibres are available on the market, some extract the target analytes by partitioning while others extract the volatile compounds by physical trapping and also by partitioning. Finally, the analytes are directly desorbed in the injector port of GC apparatus. HS-SPME is the most common and easiest technique for food quality control (Liu, Zeng, & Tian, 2004; Tat, Comuzzo, Stolfo, & Battistutta, 2005; Torrens, Riu-Aumatell, López-Tamames, & Buxareras, 2004) and in the last few years, this method has been applied to study the compounds involved in wine flavour (Bonino et al., 2003; De la Calle-García et al., 1997; Vas, Gál, Harangi, Dobó, & Vékey, 1998).

Here we analysed aroma extracts of aged cavas by means of SDE and CLSA techniques in order to identify new compounds. These extraction techniques were applied to the same cavas in order to later compare the results with qualitative data obtained by HS-SPME.

2. Materials and methods

2.1. Samples

We used a total of three cavas (Spanish sparkling wine with an ageing time of 14 months), manufactured by the same winery on an industrial scale from the autochthonous *Vitis vinifera* of the cava region. Cavas were made with a blend of the traditional white varieties: *Macabeu*, *Xarel·lo* and *Parellada* (1:1:1). Amber glass bottles of 250 mL were filled with 200 mL of cava and the headspace was saturated with carbon dioxide. The bottles were stored at 37 °C in a heater and samples were removed at 0 days (control cava) and after 30 days (aged cava) and stored at −20 °C until analysis. All glass bottles with samples were spiked with 2.5 mg L^{-1} of 2-octanol (98% purity) as internal standard (IS).

2.2. Chemicals and reagents

Ethyl propionate, ethyl butyrate, propanol, isobutyl alcohol, hexanal, isoamyl acetate, methyl hexanoate, butanol, limonene, isoamyl alcohol, ethyl hexanoate, pentanol, hexyl acetate, octanal, ethyl lactate, hexanol, *trans*-3-hexenol, *cis*-3-hexenol, methyl octanoate, ethyl octanoate, heptanol, furfural, ethyl nonanoate, octanol, γ-butyrolactone, 5-methylfurfural, methyl decanoate, ethyl decanoate, isoamyl octanoate, diethyl succinate, α-terpineol, undecanal, decanol, 2-phenylethyl acetate, ethyl dodecanoate, benzyl alcohol, hexanoic acid 2-phenylethanol, methyl tetradecanoate, octanoic acid, methyl hexadecanoate, decanoic acid, methyl octadecenoate, tetradecanoic acid, hexadecanoic acid, and 2-octanol (IS) were purchased from Sigma–Aldrich and Fluka (St. Louis, MO, USA) and showed purity higher than 95%. Individual stock standard solutions of each aroma compound were prepared by weight in methanol (SDS, Peypin, France). A commercial solution of 24 aliphatic hydrocarbons (C8–C32) in hexane (Supelco, Bellefonte, PA, USA) was added to the samples in order to calculate Kovat's indices.

2.3. Extraction methods

SDE: 50 mL of cava were placed in a 250-mL flask with 100 mL of distilled water. A second flask with 5 mL of a mixture of pentane and dichloromethane (3:1) (SDS, Peypin, France) was also attached to a Likens–Nickerson apparatus. Solvent and sample were heated to their boiling points. These temperature conditions were maintained for 4 h. A solution of polyethylenglycol in water was used as cooler to condensate vapours and to avoid loss of volatile compounds. The extract was then collected at room temperature, dried over sodium sulphate anhydrous (Panreac S.A., Montcada i Reixac, Spain) and concentrated up to 0.5 mL under nitrogen gas.

CLSA: Extraction was performed in a commercial CLSA apparatus (Brechbüler, Zurich, Switzerland). Twenty five mL of cava were diluted up to 1000 mL with double distilled water. The samples were air-stirred (flow rate of 1.5 L/min) for 70 min in a bath at 45 °C. Conditions were similar to those used by Eggers et al. (2003) in wine model solution, although in our case the adsorbent trap was a 5-mg filter of activated charcoal at 55 °C. The filter was then extracted with 40 μL of carbon disulphide (SDS, Peypin, France). The wine was diluted 1/40, so the ethanol concentration was not enough to alter the solubility of analytes. In these conditions non-saturation of activated charcoal was expected.

HS-SPME: Conditions were similar to those used by Riu-Aumatell et al. (2006). Two millilitres of sparkling wine were placed into a 10 mL glass vial (Reference 27385, Supelco, Bellefonte, PA, USA) for each HS-SPME analysis. A small magnetic stirring bar was also added. The vial was tightly capped with a PTFE septum and placed in a water bath with stirrer. The sample was maintained for 30 min at 35 °C. The fibre was activated by inserting it into the GC injector at 280 °C for 30 min. The SPME fibber was then inserted into the headspace. During the sampling time (30 min), the cava sample was stirred at constant speed (700*g*). After reaching the sampling time, the fibre was removed from the vial and inserted into the GC injection port for desorption during 5 min. A 20 mm-fibre coated with a 50/30 μm layer of divinylbenzene–carboxen–polydimethylsiloxane (DVB–CAR–PDMS) was used.

2.4. GC–MS analysis

Volatiles were identified on a mass spectrometer Agilent Technologies 5973 Network coupled directly online to an Agilent Technologies 6890N Network GC System (both, Palo Alto, CA, USA). Spectra were obtained on electron impact at 70 eV, scanning from 15 to 250 m/z at 2 scans s^{-1}. The GC system was equipped with a supelcowax 10 (Supelco, Bellefonte, PA, USA) capillary column with a 20 M polyethyleneglycol stationary phase (30 m × 0.25 mm × 0.25 μm) and SPB-1 (Supelco, Bellefonte, PA, USA) fused silica capillary column (30 m × 0.25 mm × 0.25 μm). The temperature programme ranged from 40 °C to 250 °C in the following way: 40 °C for 10 min, from 40 °C to 200 °C at 2 °C min^{-1}, one min at 200 °C, from 200 °C to 250 °C at 2 °C min^{-1}, and finally, 10 min at 250 °C. The carrier gas was helium at 1 ml min^{-1}. One microlitre of each extract from SDE and CLSA was injected in splitless mode. An 8-min solvent delay was programmed when the SDE extracts were injected.

2.5. Identification

Volatiles were identified by comparing the retention index and mass spectrum of commercial standards when they were available. The volatile compounds were also identified using the software library of mass spectra database Willey 6.1 (NY, USA); moreover, they were identified using theoretical retention index calculation on polar column (Supelcowax-10 described above) and on non-polar column (SPB-1 described above). In addition, another GC–MS integrated quadrupole Trace MS Plus apparatus (ThermoElectron, USA) was used to confirm the identification of the volatile compounds. The following temperature programme was applied: 35 °C for 5 min, up to 250 °C at 5 °C min^{-1}, and finally, 20 min at 250 °C. A DB-5 column (60 m × 0.25 mm × 0.25 μm) (J & W scientific, Folsom, CA, USA) was used with this GC–MS system. The MS parameters were: a mass range of 35–350 m/z, with 0.5 scan s^{-1}, and ionization energy of 70 eV. NIST/EPA/NIH mass spectral database 1998 (Gaitherfburg, MD, USA) library was used with this GC–MS equipment.

3. Results and discussion

Three extraction methods (SDE, CLSA, and HS-SPME) were used to identify volatile compounds in cava (Table 1). The compounds are listed following elution order, and including their chemical name, CAS number (SciFinder Scholar® 2006) or mass fragments, Kovat's index for polar and non-polar columns, cited Kovat's and the identification method used. Chromatograms from SDE, CLSA, and HS-SPME are shown in Fig. 1. The peak numbers of the chromatograms match the numbers of Table 1. The semi-quantitative analysis was performed by internal normalization and is shown in Table 1 by means of an asterisk scale (<1%; 1–10%, and >10%).

SDE required 50 mL of sample for extraction while CLSA and SPME needed 25 and 2 mL, respectively. Therefore, SDE allowed us to obtain a cava chromatogram with a wider volatile profile than those from CLSA and SPME (Fig. 1). However, SDE is a lengthy process (4 h); moreover, this method requires solvents and temperature for volatile compounds extraction. In this study we used dichloromethane because of its polarity. Consequently, high extraction capacity for polar volatile compounds (alcohols, aldehydes, and acids) was obtained. This capacity was not observed in CLSA and SPME because these methods were based on the equilibrium partitioning of the volatiles between solution phase (sparkling wine) and vapour or gas phase. This equilibrium is regulated by the partition coefficient and thus it is difficult to apply HS-SPME and CLSA for the extraction of polar compounds from a hydro-alcoholic matrix like cava. CLSA (stripping technique) and HS-SPME identified only the most abundant alcohols such as propanol, isopropanol, isoamyl alcohol, hexanol, *cis*-3-hexenol and 2-phenylethanol. In contrast, in SDE extracts some ramified alcohols, like 2-methyl-1-pentanol, 3-methyl-1-pentanol, and isohexanol (4-methyl-1-pentanol) were detected (Table 1). These alcohols have been described in wine (Tat et al., 2005), sherry (Zea et al., 2001), and cognac (Ledauphin et al., 2005), and their occurrence could be related to oxidative ageing (Zea et al., 2001). Moreover, tentatively identified 3-ethoxy-1-propanol in the SDE extracts has been reported in

Table 1
Volatile compounds identified by SDE, CLSA, and HS-SPME

		Cas No./MS^{A}	IK CW^{B}	Cited IK^{C}	IK SPB1 ^{D}	ID^{E}	Control cava			Aged cava		
							SDE	CLSA	SPME	SDE	CLSA	SPME
1	Ethyl propanoate	105-37-3	<1000			f, g		*	*		*	*
2	Ethyl isobutyrate	97-62-1	<1000	965^{F}		g, h		*	*		*	*
3	Butyraldehyde, diethyl acetal	3658-95-5	<1000	1031^{G}		g, h	*			*		
4	Ethyl butyrate	105-54-4	1031	1052^{H}	800	f, g, h	*	*	*	*	**	*
5	Propanol	71-23-8	1046	1069^{H}		f, g, h	**		*	**		*
6	Ethyl 2-methylbutanoate	7452-79-1	1055	1052^{F}	820	g, h		*	*		*	*
7	Ethyl isovalerate	108-64-5	1061	1070^{F}	829	g, h	*	*	*		*	*
8	Isobutyl alcohol	78-83-1	1108	1099^{H}		f, g, h	**	*	*	**	*	*
9	Valeraldehyde, diethyl acetal	3658-79-5	1120	1135^{G}		g, h	*			*		
10	Isovaleraldehyde, diethyl acetal	03842-03-3	1079	1062γ		g, h	*			*		
11	Hexanal	66-25-1	1094	1064^{G}		f, g, h	*					
12	Isoamyl acetate	123-92-2	1123	1142^{H}	851	f, g, h	*	**	*	*	**	*
13	Methyl hexanoate	106-70-7	1101	1176^{G}	906	f, g, h		*	*		*	
14	Butanol	71-36-3	1159	1147^{G}		f, g, h	*			*		
15	1-Pentene-3-ol	918-85-4	1174	1157^{I}		g, h	*			*		
16	Limonene	138-86-3	1193	1175^{G}	1030	f, g, h	*					
17	Isoamyl alcohol	123-51-3	1210	1211^{J}	801	f, g, h	***	***	**	***	***	**
18	Ethyl hexanoate	123-66-0	1232	1239^{H}	977	f, g, h	*	***	***	*	***	***
19	Isopropyl disulfide	4253-89-8	1248			g	*					
20	Hexanal, diethyl acetal	3658-93-3	1250	1235^{G}		g, h	*					
21	Pentanol	71-41-0	1258	1249^{G}		f, g, h	*					
22	Hexyl acetate	142-92-7	1269	1267^{J}	1012	f, g, h		**	*		*	*
23	2-Octanone	111-13-7	1278	1278^{I}	962	g, h	*	*	*	*	**	*
24	Ethyl 3-hexenoate	2396-83-0	1286	1291^{G}		g, h		*	*		*	
25	Octanal	124-13-0	1290	1282^{G}	973	f, g, h	*					
26	Isohexanol	626-89-1	1317	1312^{G}	838	g, h	*			*		
27	2-Penten-1-ol	20273-24-9	1321			g	*					
28	1-Pentanol, 3-methyl-	589-35-5	1329	1323^{G}	852	g, h	*	*		*	*	
29	Heptanal, diethyl acetal	688-82-4	1334	1332^{G}		g, h	*					
30	Ethyl 2-hexenoate	1552-67-6	1336			g		*			*	
31	2-Methyl-1-pentanol	105-30-6	1337		837	g	*					
32	Ethyl lactate	97-64-3	1348	1367^{H}	821	f, g, h	**	*	*	**	*	
33	Hexanol	111-27-3	1351	1371^{H}	845	f, g, h	*	**	*	**	**	*
34	*trans* 3-Hexenol	928-97-2	1367	1385^{H}	854	f, g, h	*			*		
35	3-Ethoxy-1-propanol	111-35-3	1375		840	g	*			*		
36	*cis*-3-Hexenol	928-96-1	1379	1401^{J}	853	f, g, h	*	*	*	*	*	*
37	Methyl octanoate	111-11-5	1383	1386^{G}	1103	f, g, h	*	*	*		*	*
38	Ethyl octanoate	106-32-1	1430	1436^{J}	1178	f, g, h	**	***	***	*	***	***
39	Heptanol	111-70-6	1458	1454^{G}	957	f, g, h	*	*		*	*	*
40	Furfural	98-01-1	1459	1474^{F}	828	f, g, h	*	*	*	*	*	*
41	2-Ethylhexen-1-ol	29594-61-4	1478			g		*			*	
42	2-Acetylfuran	1192-62-7	1490	1500^{G}	892	g, h	*					
43	Vitispirane 1	65416-59-3	1508	1507^{J}	1248	g, h	*	*	*	*	*	*
44	Vitispirane 2	65416-59-3	1511	1510^{G}	1252	g, h	*	*	*	*	*	*
45	Methylthiophen-3-one	13679-85-1	1519	1538^{G}	937	g, h	*			*		
46	Nonanal, diethyl acetal	54815-13-3	1530			g	*					
47	Ethyl propionate	123-29-5	1536	1530^{G}		f, g, h	*					

(*continued on next page*)

Table 1 (*continued*)

		Cas No./MS[A]	IK CW[B]	Cited IK[C]	IK SPB1 [D]	ID[E]	Control cava			Aged cava		
							SDE	CLSA	SPME	SDE	CLSA	SPME
48	1-Octanol	111-87-5	1561	1559[G]	870	f, g, h	*	*	*	*	*	*
49	γ-Butyrolactone	96-48-0	1568	1634[I]	835	f, g, h	*					
50	5-Methylfurfural	620-02-0	1572	1566[G]	926	f, g, h	*			*		
51	Diethyl malonate	105-53-3	1582		1043	g	*			*		
52	Methyl decanoate	110-42-9	1593	1586[G]	1302	f, g, h	*	*	*	*		*
53	Ethyl 2-furancarboxylate	614-99-3	1621	1621[G]	1009	g, h	*	*		*	*	
54	Ethyl decanoate	110-38-3	1639	1678[H]	1376	f, g, h	*	**	**	*	*	**
55	Isoamyl octanoate	2035-99-6	1652	1649[J]	1429	f, g, h		*	*			
56	Diethyl succinate	123-25-1	1678	1662[J]	1146	f, g, h	**	**	*	**	**	*
57	Ethyl 9-decenoate	67233-91-4	1691	1681[J]	1360	g, h	*	*	**	*	*	*
58	Terpene compound (ms)	93, 109, 204	1699			g	*			*		
59	α-Terpineol	7785-53-7	1710	1694[G]	1185	f, g, h				*		
60	Terpene compound (ms)	105, 161, 204	1717			g				*		
61	Terpene compound (ms)	93, 161, 204	1723		1468	g				*		
62	TDN	30364-38-6	1731	1719[J]	1313	g, h	*		*	*		*
63	Undecanal	112-44-7	1747			g	*					
64	Terpene compound (ms)	119, 132, 202	1756		1456	g	*			*		
65	1-Decanol	112-30-1	1765	1764[G]	1263	f, g, h		*	*		*	
66	2-Phenylethyl acetate	93-92-5	1811	1795[J]	1222	f, g, h	*	*		*	*	
67	Ethyl dodecanoate	106-33-2	1827	1833[J]	1576	f, g, h		*	*			
68	Benzyl alcohol	100-51-6	1870	1881[G]	1033	f, g, h	*					
69	Hexanoic acid	142-62-1	1885	1826[J]	1007	f, g, h	**		*	**		*
70	2-Phenylethanol	60-12-8	1906	1888[J]	1074	f, g, h	**	**	*	**	**	**
71	Isopropyl myristate	110-27-0	1936	1964[H]		g, h		*			*	
72	Methyl tetradecanoate	124-10-7	2009		1682	f, g	*					
73	Lilial	80-54-6	2037			g				*		
74	Ethyl tetradecanoate	124-06-1	2049	2046[G]	1779	f, g, h	*					
75	Octanoic acid	124-07-2	2087	2038[J]	1164	f, g, h	**	**	**	*	*	**
76	*p*-Vinylguaiacol	7786-61-0	2192	2200[G]	1274		*					
77	Methyl hexadecanoate	112-39-0	2216	2213[G]	1909	f, g, h	*			*		
78	Terpene compound (ms)	119, 161, 204	2234			g	*			*		
79	α-Amyl-cinnamaldehyde	122-40-7	2247		1598	g	*			*		
80	Ethyl hexadecanoate	628-97-7	2255	2229[J]	1979	f, g, h	*			*		
81	Decanoic acid	334-48-5	2339	2254[J]	1349	f, g, h	**	***		**	*	
82	Methyl octadecenoate	27234-05-5	2390	2417[G]		f, g, h				*		
83	Tetradecanoic acid	544-63-8	>2600	>2600[G]	1731	f, g, h	*			*		
84	Hexadecanoic acid	57-10-3	>2600		1919	g	*			*		

The semi-quantitative analysis was performed by internal normalization and it was expressed by means of an asterisk scale (<1%; 1–10%, and >10%).

f: identification based on retention time and electron impact mass spectrum of standards; g: identification based on examination of electron impact mass spectrum; h: identification based on theoretical retention index calculation.

[A] CAS number or mass fragments of identified compounds.

[B] Kovat's indices on Supelcowax-10 column.

[C] Kovat's indices of polar columns reported in literature.

[D] Kovat's indices on SPB-1 column.

[E] Identification method.

[F] Aznar et al. (2001).

[G] Ledauphin et al. (2005).

[H] Câmpeanu et al. (1998).

[I] Comuzzo et al. (2006).

[J] Riu-Aumatell et al. (2006).

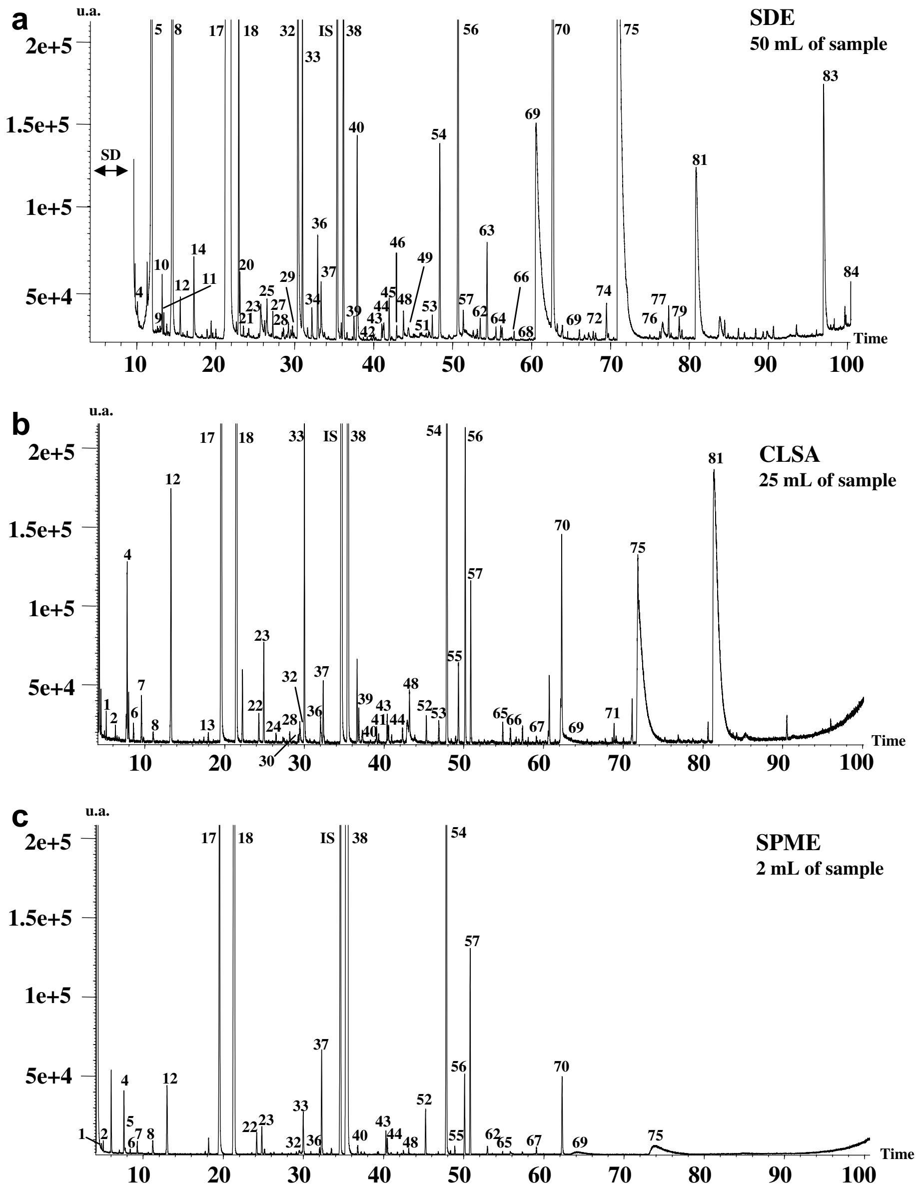

Fig. 1. Chromatograms of sparkling wines: (a) chromatogram of a sparkling wine extract obtained by SDE; (b) chromatogram of a sparkling wine extract obtained by CLSA; (c) chromatogram of a sparkling wine obtained by HS-SPME. Peaks numbers are identified according to Table 1.

wine that has undergone malolactic fermentation (Fernandes, Relva, Gomes da Silva, & Costa Freitas, 2003). Finally, two sulphur compounds (isopropyl disulfide and methylthiophen-3-one) were detected in the SDE extracts. The presence of methionine and yeast metabolism could explain the formation of these sulphur compounds (Bayonove, Baumes, Crouzet, & Günata, 2000; Blaise & Bertrand, 2000). The highest amounts of polar compounds were obtained by SDE; however, HS-SPME showed the most representative polar compounds of cava. Indeed, it may be a useful extraction method for alcohols and acids. However, the high extraction capacity of SDE and CLSA for acid compounds (hexanoic, octanoic and decanoic acids) may mask the detection of other minority compounds as a result of peak tailing (Fig. 1, peaks 69, 75, 81).

Ethyl esters of aliphatic acids are an important aromatic family in the volatile profile of sparkling wines. Although SDE, CLSA, and HS-SPME were suitable methods for determining ethyl esters (ethyl hexanoate, ethyl octanoate, and ethyl decanoate) in cava samples, SPME was the fastest and easiest technique to extract the main esters of volatiles. Other ethyl esters detected using these three extraction methods were ethyl 9-decenoate, ethyl lactate and diethyl succinate. The first ethyl ester has been reported in sparkling wine samples (Riu-Aumatell et al., 2006), and in white wines (Câmpeanu et al., 1998). Ethyl lactate and diethyl succinate are post-fermentative volatiles formed during the ageing of cava in contact with lees from the second fermentation. The concentration of diethyl succinate increases during ageing and this compound is a marker of the evolution of cava during cellar storage (Francioli, Guerra, Lopez-Tamames, Guadayol, & Caixach, 1999; Riu-Aumatell et al., 2006).

Other ester compounds present in the volatile profile of cava were methyl esters of hexanoic, octanoic, and decanoic acids. Ledauphin et al. (2005) detected methyl esters in spirit distilled beverages. In these beverages, the formation of methyl esters is due to the high temperature required during the distillation step of *cognac* elaboration. However, in the current study, the formation of these methyl esters was not related to artefacts formed as a result of the temperature used during extraction by SDE, because they were detected with SDE, CLSA, and HS-SPME techniques. Moreover, methyl esters in wines are related to yeast fermentation (Castro et al., 2004).

Sparkling wine acetates decrease along ageing time of cava in contact with lees (Riu-Aumatell et al., 2006). The samples used in our study were aged for 14 months. As a result, only isoamyl acetate, hexyl acetate, and 2-fenilethyl were detected. CLSA shows better extraction capacity for acetates than HS-SPME and SDE. However, the main acetate in wine (isoamyl acetate) was detected with all three techniques.

Acetal formation was attributed to the reaction between aldehydes and ethanol by temperature or oxidation mechanisms. We found that CLSA and HS-SPME did not detect acetals; therefore we propose that acetals were artefacts formed as a result of the high extraction temperature required when applying SDE.

Terpene compounds (lilial and compound numbers 58, 60, 61, 64, and 78) were better determined by SDE than by CLSA or HS-SPME (Fig. 1 and Table 1). Two characteristic norisoprenoids of aged cava, vitispirane isomers and TDN, were identified with all the methods tested. These compounds were markers of ageing because their concentrations increased with ageing (Francioli et al., 1999; Riu-Aumatell et al., 2006). Terpene and norisoprenoid compounds are varietal volatiles released during ageing, and will be better characterized by SDE.

Furfurals formed by sugar degradation in the presence of oxygen are usually found in sweet wines that have gone through biological ageing (Schneider et al., 1998). SDE and CLSA showed a greater capacity to determine ethyl 2-furancarboxylate, 2-acetylfuran, and 5-methylfurfural in cava samples than HS-SPME.

4. Conclusion

Our findings contribute to a better knowledge of the volatile constituents of cava sparkling wine. Eighty-four compounds were identified using SDE, CLSA, and HS-SPME extraction methods. SDE showed the best extraction rates for all families of volatiles. However, this laborious technique is costly in solvents and time. A good alternative is CLSA because it provides a wide profile of volatile compounds in cava samples. However, CLSA is also lengthy. HS-SPME is a straightforward and fast extraction method which does not require sample treatments. This method allowed us to obtain the most important volatile compounds of each chemical family. Further studies will focus on HS-SPME to assess the occurrence of compounds formed from sugar degradation (furfurals and carbonyl compounds) during biological ageing of sparkling wine.

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