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Development of volatile compounds of cava (Spanish sparkling wine) during long ageing time in contact with lees

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Abstract

In order to study the evolution of volatile compounds during long ageing times in contact with lees (more than 2 years), Headspace–solid phase microextraction (HS/SPME) method was applied to different cavas (Spanish Sparkling wine). Two different fiber coatings were used: PDMS which extracts the analytes by partitioning and DVB–CAR–PDMS which extracts either by partitioning or physically trapped the target compounds. Even though the volatile profile obtained by gas chromatography/mass spectrometry (GC/MS) was similar, the triple phase seems to be more suitable to follow the development of the volatile profile during its ageing time. Hexyl, 2-phenylethyl and isoamyl acetates significantly decrease over time, while 1,2-dihydro-1,1,6-trimethylnaphthalene (TDN), vitispirane and diethyl succinate significantly increase during ageing in contact with lees.

Keywords: Ageing; Cava; Headspace/solid-phase microextraction; Volatile compounds

1. Introduction

Cava is the Spanish sparkling wine (Certified Brand of Origin) produced by the traditional method which consists of a second fermentation in closed bottles and ageing in contact with lees for at least 9 months, the minimum time legally established (Council Regulation (EC), 1493/1999). Empirically, cava makers believe that long ageing time provides high quality cavas with a complex bouquet. This aroma is difficult to standardize due to the influence of biological processes, such as yeast autolysis (de la Presa-Owens, Schlich, Davies, & Noble, 1998; Escudero, Charpentier, & Etievant, 2000; Leroy, Charpentier, Duteurtre, Feuillat, & Charpentier, 1990; Loyaux & Adda, 1981; Pozo-Bayón, Polo, Martín-Álvarez, & Pueyo, 2004; Tominaga, Guimbertau, & Dubordieu, 2003). The characterization of the development with to the ageing time of the bouquet of the high quality cavas could provide a great economy of time and money for the wine makers.

Very few studies exist on the volatile composition of cava according to the ageing time in contact with lees (Francioli, Guerra, López-Tamames, Guadayol, & Caixach, 1999; Francioli, Torrens, Riu-Aumatell, López-Tamames, & Buxaderas, 2003). These authors suggested that some volatile compounds, determined by the solid phase microextraction technique (SPME) using the polydimethylsiloxane (PDMS) fiber, characterized cavas with different ageing times. The PDMS fibers used previously for cava (Francioli et al., 1999, 2003) is a nonpolar fiber which extracts the target analytes by partitioning. Actually, different commercial bipolar fiber coatings exists. In these bipolar phase coatings, the volatile compounds could be extracted either by partitioning or they could be physically trapped (Shirey & Mindrup, 1999). Recently, in the study of Torrens,

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Riu-Aumatell, López-Tamames, and Buxaderas (2004), four fiber coatings were applied for white and red wine, and the divinylbenzene–carboxen–polydimethylsiloxane (DVB–CAR–PDMS) was shown to be the most appropriate to obtain the wider wine volatile profile.

In the current research, we studied the development of post-fermentative aroma and its evolution in cavas during a long ageing time in contact with lees (more than 2 years). The evolution of the volatile profile of two cava series produced from different grape varieties after 9–27 months of ageing was performed by HS/SPME and GC/MS every three months. The extraction was performed with two fibers PDMS, as proposed Francioli et al. (2003), and the triple phase DVB–CAR–PDMS as recommended for Torrens et al. (2004) in wines to consider if the volatile profile obtained differs according to the fiber used.

2. Material and methods

2.1. Samples

Two series of cava (Spanish sparkling wine), with ageing time of 9, 12, 15, 18, 21, 24, and 27 months, were made by the same winery at industrial scale, from the autochthonous *Vitis vinifera* of the cava region. One series was a blend of the traditional white varieties to produce cava: Macabeu, Xarel·lo and Parellada (1:1:1). The other series was a *rosé* cava of the red variety, Trepat.

2.2. Reagents

An internal standard solution (IS) of nonanoic acid ethyl ester, 95% purity (Sigma, St. Louis, MO, USA) in methanol (SDS, Peypin, France) was prepared at a concentration of 1 g/l.

2.3. SPME fiber coatings

The SPME fibers tested were 100 μm polydimethylsiloxane layer (PDMS) recommended for volatiles and 10 mm fiber coated with 50/30 μm divinylbenzene–carboxen–polydimethylsiloxane layer (DVB–CAR–PDMS) recommended for flavours (volatiles and semivolatiles) in the catalog recommendations of Supelco (Supelco, Bellefonte, PA, USA).

2.4. SPME conditions

SPME was performed at 35 °C in the headspace mode (with a distance from the liquid surface of 20 mm) in closed 10 ml vials (Reference 27385, Supelco, Bellefonte, PA, USA) containing 5 ml of sample and 5 μl of IS for 30 min with magnetic stirring (700*g*). The fiber was activated prior to use each day by inserting it into the GC injector at 250 °C for 30 min. The desorption time was 5 min. For each cava sample HS–SPME was applied in triplicate.

2.5. GC–MS analysis

The separation was carried out by gas chromatography/mass spectrometry (GC/MS) using a Hewlett-Packard (Palo Alto, CA, USA) 5971A mass spectrometer coupled to a Hewlett–Packard (Palo Alto, CA, USA) 5890A Series II chromatograph. A Supelcowax 10 (Supelco, Bellefonte, PA, USA) capillary column with polyetilenglycol 20 M stationary phase (30 m × 0.25 mm, 0.25 μm) and SPB-1™ capillary column with fused silica stationary phase (30 m × 0.25 mm, 0.25 μm) were used. The chromatographic conditions used were the same as that of Francioli et al. (2003). Electron impact mass spectra were recorded at a voltage of 70 eV ionization energy in the 15–250 u mass range, and 2 scans/s.

2.6. Qualitative analysis

Fig. 1 showed the chromatograms of 9 and 27 months of ageing time obtained with PDMS (a and c) and DVB–CAR–PDMS (b and d), respectively. The number of compounds corresponds to the compounds listed in Table 1. Volatile compounds were identified by comparing them with a private spectra library created with chemical standards and two spectral libraries (NIST/EPA/MSDC 49K Mass Spectral Database, Hewlett–Packard Co., Palo Alto, CA, USA and Registry of Mass Spectral Data with Structures, Wiley 6.1, NY, USA), as well as with relative retention times when chemical standards were available. We also used the retention index standards (Sigma, St Louis, MO, USA) of C8 and C32 aliphatic hydrocarbons dissolved in methanol to calculate the Kováts index (KI) in CW and SPB-1 columns, respectively (Table 1).

2.7. Quantitative analysis

Quantification was done by the internal standard method and the volatile compounds identified were quantified by considering the relative response factor to be 1 and were expressed as mg/l equivalents of IS. The RSD values, calculated from the triplicates of each cava sample, were similar for both fibers used (data not shown).

2.8. Statistical procedures

Principal components analysis (PCA) was applied in order to find either varietal, ageing time or fiber coating groupings. The relationship between ageing time (months) and the levels of aroma compounds was made by simple regression analysis and one-way ANOVA

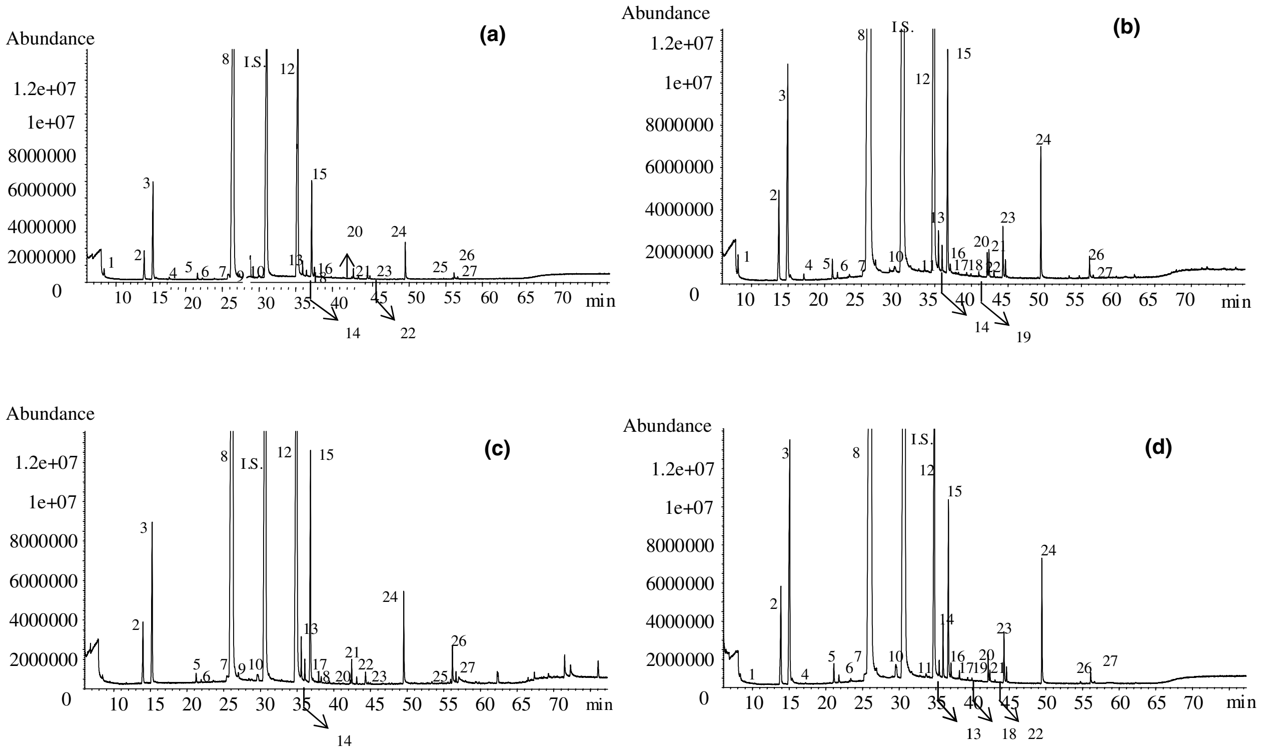

Fig. 1. Chromatograms of sparkling wines by HS/SPME method: (a) chromatogram of a sparkling wine obtained with PDMS fiber of 9 months of ageing time; (b) chromatogram of a sparkling wine obtained by DVB–CAR–PDMS fiber of 9 months of ageing time; (c) chromatogram of a sparkling wine obtained with PDMS fiber of 27 months of ageing time; (d) chromatogram of a sparkling wine obtained by DVB–CAR–PDMS fiber of 27 months of ageing time. Peak numbers correspond to the compounds listed in Table 1.

analysis. Significant result was considered when $p < 0.05$ (Statgraphics Plus 4.1).

3. Results and discussion

Twenty seven compounds were detected in the characteristic volatile profile of cavas (Fig. 1). The differences observed according to the fiber used (Fig. 1(a) and (c) with Fig. 1(b) and (d)) or the ageing time (Fig. 1(a) and (b) with Fig. 1(c) and (d)) were mainly quantitative. The numbered peak 11 (unknown peak 1, *m/z* 59, 72, 88, 101) was only detected with the triple phase fiber while the isoamyl hexanoate was only detected with the PDMS phase (Table 1). The tentatively identified volatile compounds vitispirane, 1,2-dihydro-1,1,6-trimethylnaphthalene (TDN) ethyl 2-methyl octanoate and ethyl decenoate (Table 1 and Fig. 1) were previously cited by Francioli et al. (1999) in sparkling wines. These compounds and ethyl hexadecenoate were detected with both fibers.

In order to obtain the groupings of the cavas according to the volatile compounds determined by HS/SPME, principal components analysis (PCA) was carried out (Fig. 2). Two groups were obtained according to the fiber used [Component 1 (34%)]. The analysis of variance (one-way ANOVA) shows significant differences between these two groups (data not shown). Ethyl esters of high molecular weight (ethyl 2-methyloctanoate, dodecanoate, hexadecanoate and hexadecenoate) and isoamyl hexanoate were significantly higher in the samples determined by PDMS fiber while ethyl esters of low molecular weight (ethyl hexanoate, octanoate and lactate), alcohols (hexanol and 2-phenylethanol), fatty acids (hexanoic and octanoic acid) and post-fermentative aromas (vitispirane and diethyl succinate) were higher in cavas determined by DVB–CAR–PDMS. The two action mechanism of the triple phase fiber may have favoured the extraction of more polar compounds such as alcohols and acids.

When the principal components analysis (PCA) was performed with both fibers separately, any significant grouping according to the ageing time was obtained with the PDMS fiber (data not shown). Probably the combination of both mechanisms (absorbent and adsorbent) in the triple phase fiber enhanced its discriminant capacity. The PCA of the volatile compounds obtained with DVB–CAR–PDMS fiber is shown in Fig. 3. Although the volatile composition was different in white or rosé sparkling wine [Component 1 (32%)], the samples were distributed on the plane according to their ageing time [Component 2 (20%)]. Samples between 9 and

Table 1
Compounds determined by the HS/SPME method, Kováts index, identification and minimum and maximum values for the fibers PDMS and DVB–CAR–PDMS

		KI[a] Cw[b] phase	KI SPB- 1[c] phase	Id.[d]	PDMS		DVB–CAR–PDMS	
					Minimum[e]	Maximum[e]	Minimum	Maximum
1	Isoamyl acetate	1116	860	A, B	n.d.[f]	2.799	n.d.	5,877
2	Isoamyl alcohol	1211	719	A, B	0.048	1.612	0.023	0.855
3	Ethyl hexanoate	1230	984	A, B	0.594	2.242	0.047	2.655
4	Hexyl acetate	1267	1012	A, B	n.d.	0.684	0.003	0.033
5	Ethyl lactate	1334	1135	A, B	0.021	0.165	0.023	0.152
6	Hexanol	1347	858	A, B	n.d.	0.066	0.022	0.059
7	Ethyl 2-methyloctanoate	1415	1224	B[g]	n.d.	0.245	n.d.	0.120
8	Ethyl octanoate	1436	1184	A, B	1.427	26.652	8.600	22.972
9	Isoamyl hexanoate	1451	1233	A, B	n.d.	0.203	n.d.	n.d.
10	Vitispirane	1507	1260	B[g]	n.d.	0.126	0.020	0.223
11	Unknown peak 1 (*m*/*z* 59, 72, 88, 101)	1603	1147		n.d.	n.d.	0.015	0.099
12	Ethyl decanoate	1634	1381	A, B	3.314	9.990	3.043	8.630
13	Isoamyl octanoate	1649	1429	A, B	0.061	0.397	0.083	0.344
14	Diethyl succinate	1662	1153	A, B	0.004	0.205	0.089	0.460
15	Ethyl decenoate	1681	1437	B[g]	n.d.	2.241	0.729	10.449
16	Unknown peak 2 (*m*/*z* 57, 97, 151, 180)	1689	1247		n.d.	1.086	0.010	0.125
17	1,2-Dimethyl-1,1,6-trimethylnaphthalene	1719	1474	B[g]	n.d.	0.063	n.d.	0.068
18	Unknown peak 3 (*m*/*z* 55, 70, 83, 97)	1750	1263		n.d.	0.035	0.007	0.041
19	2-Phenylethyl acetate	1795	1222	A, B	n.d.	0.048	n.d.	0.080
20	Hexanoic acid	1826	1579	A, B	n.d.	0.162	0.047	0.204
21	Ethyl dodecanoate	1833	1577	A, B	0.021	0.606	0.042	0.135
22	Isoamyl decanoate	1853	1633	A, B	n.d.	0.076	0.002	0.081
23	2-Phenylethanol	1888	1080	A, B	n.d.	0.385	0.157	1.202
24	Octanoic acid	2038	1356	A, B	n.d.	0.691	0.250	1.639
25	Ethyl hexadecanoate	2229	1978	A, B	n.d.	0.199	n.d.	0.026
26	Decanoic acid	2254	1363	A, B	0.016	0.619	0.005	1.527
27	Ethyl hexadecenoate	2269	1438	B[g]	n.d.	0.207	n.d.	0.018

[a] Kováts index.
[b] Carbowax phase.
[c] Silicone phase.
[d] Identification (A, retention time; B, mass spectrometry).
[e] mg/l equivalents of ethyl nonanoate (IS).
[f] Not detected.
[g] Tentatively identified.

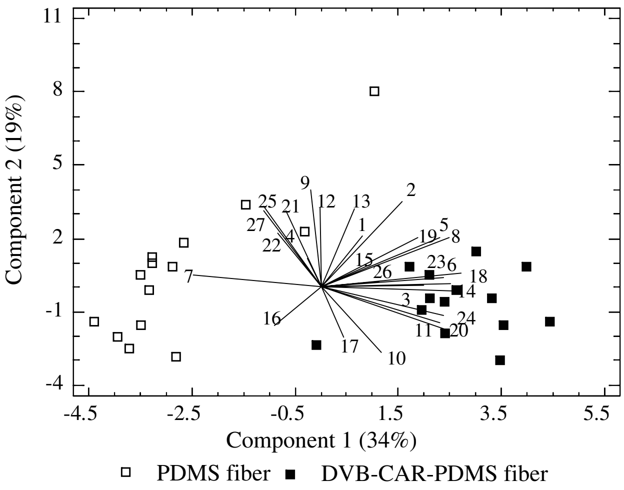

Fig. 2. Principal components analysis of the mean of the triplicates of the volatile compounds of cavas determined by PDMS and DVB–CAR–PDMS fibers. The vector numbers correspond to that of Table 1.

15 months were in the lower zone of the plane while the samples of 21 to 27 months were in the upper zone. Some acetate, ethyl and isoamyl esters of high molecular weight, seem to be typical aromas of cavas of low ageing time, while vitispirane, diethyl succinate, TDN, hexanol (related to autolysis process according to Francioli et al., 2003) and ethyl lactate seem to be compounds inherent in the bouquet of long aged cavas ($p < 0.05$) (Francioli et al., 1999; Francioli et al., 2003). Similar results were obtained by Loyaux and Adda (1981) during Champagne ageing, which showed a decrease in hexyl acetate and an increase in the concentration of vitispirane. Some authors suggest that it is the yeast autolysis, that occurs after approximately 18 months in contact with lees, which causes changes in aroma composition due to the enzymatic release from glycosidic precursors. Carotenoids are the origin molecules of C_{13} norisoprenoids. These compounds are substances that could originate from the direct degradation of carotenoid molecules

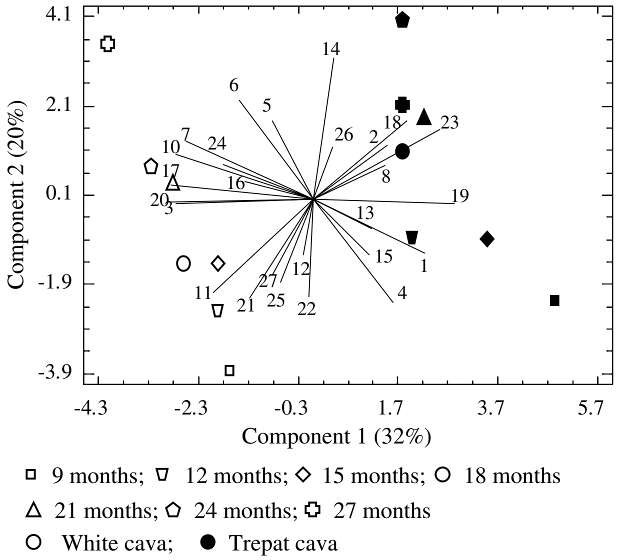

Fig. 3. Principal component analysis of the mean of the triplicates of the volatile compounds determined by DVB–CAR–PDMS fiber. The vector numbers correspond to that of Table 1.

and also from the hydrolysis of glycoside molecules. Vitispirane has a megastigme precursor and is linked to a sugar molecule. TDN is released during bottle storage and its origin is not clear. While some authors have shown that TDN is a direct degradation product of carotene (Rapp, 1998), others have shown two possible precursors in grape linked to a sugar molecule (Winterhalter, 1991; Silva Ferreira & Guedes de Pinho, 2004).

These changes of volatile composition according to ageing time, were confirmed by simple regression analysis. Table 2 shows the coefficients of regression (r) and the significant levels (p) obtained between the aroma compounds and the ageing time (months) according to the fiber used and the cava type (white and rosé). Even though most of the regression models were linear ($y = a + bx$), some of them follow a reciprocal model ($y = a + b/x$). This last model was followed for some compounds determined with triple phase fibers notably ethyl hexadecanoate in white cava and isoamyl acetate and ethyl decenoate in Trepat cava. More significant results were obtained with triple phase than PDMS fiber. The evolution of acetate esters decreased with the ageing time (Fig. 3) while vitispirane, TDN, and diethyl succinate increased with time in contact with lees (Fig. 3). It was noticeable that TDN only was found in white cava. Probably the precursor of TDN had a varietal origin. Our results could confirm the presence of TDN precursor according to the varietal base wine. This precursor could be an early norisoprenoid as 3,6-dihydroxy-7,8-dihydro-α-ionone, 3,4-dihydroxy-7,8-dihydro-β-ionone and 3,9-dihydroxyteaspirane (Winterhalter, 1993), could be linked to a sugar molecule or TDN could be a compound resulting from carotene metabolism by yeast.

In conclusion, some volatiles showed a significant behaviour in relation to ageing in contact with lees (more than 2 years). Acetate esters were higher in young cavas while TDN, vitispirane and diethyl succinate increased in long aged samples. Despite the qualitative volatile profile obtained being similar with both fibers, DVB–CAR–PDMS fiber could be more suitable than PDMS to follow the volatile kinetics of cava bouquet. A lot of questions still exist according to the origin of post-fermentative aromas in bottle ageing in contact with lees. The origin of aromatic compounds in aged cavas (Spanish sparkling wine) is currently under investigation.

Table 2
Significant results of regression analysis between the ageing time (months) and aroma compounds for fiber type and cava type

	PDMS				DVB–CAR–PDMS			
	White cava ($n = 7$)		Trepat cava ($n = 7$)		White cava ($n = 7$)		Trepat ($n = 7$)	
	r	p	r	p	r	p	r	p
Isoamyl acetate	−0.858	<0.05	−0.754	<0.05	−0.773	<0.05	−0.864[c]	<0.05
Hexyl acetate	−0.872	<0.01	−0.417	<0.05	−0.881	<0.01	−0.945	<0.005
Ethyl lactate	n.s.[a]		n.s.		n.s.		0.830	<0.05
Hexanol	n.s.		n.s.		0.880	<0.01	0.473	<0.05
Vitispirane	0.824	<0.05	0.900	<0.005	0.990	<0.001	0.730	<0.1
Unknown peak 1	n.d.[b]		n.d.		−0.831	<0.05	n.s.	
Diethyl succinate	0.848	<0.01	n.s.		0.940	<0.005	0.898	<0.01
Ethyl decenoate	n.s.		n.s.		n.s.		−0.776[c]	<0.05
1,2-Dimethyl-1,1,6-trimethylnaphthalene	0.899	<0.005	n.d.		0.971	<0.001	n.d.	
2-Phenylethyl acetate	n.d.		−0.625	<0.01	−0.807	<0.05	n.s.	
Ethyl dodecanoate	n.s.		n.s.		−0.917	<0.005	−0.443	<0.05
Ethyl hexadecanoate	n.s.		n.s.		0.888[c]	<0.01	n.d.	

[a] Not significative.
[b] Not detected.
[c] Reciprocal-X model.

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