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Comparison of volatile compounds during biological ageing and commercial storage of Cava (Spanish sparkling wine): The role of lees

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A R T I C L E I N F O

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A B S T R A C T

Cava is a sparkling wine produced using a traditional method that must be aged in contact with lees for a minimum period of nine months. The contact between wine and lees improves the quality of the final product, and aroma is one of the most important qualitative parameters of a wine. The aim of the work was to study the role of lees in the ageing of Spanish sparkling wine (Cava), by sampling at industrial scale the bottles, from 9 to 30 months of ageing, jointly with the winery and in real time. The volatile profile of Cava during biological ageing and commercial storage, after disgorging, was evaluated by Headspace-Solid Phase Microextraction coupled to gas chromatography–mass spectrometry. More than 60 compounds were identified from several chemical classes including esters, alcohols, terpenes, furans, norisoprenoids, and fatty acids. A reduction in volatile components was observed when the disgorging step took place. When the behaviour of aroma over time was assessed, the principal factor that discriminated between samples was the type of ageing i.e. with or without lees. Evaluation of volatiles over time revealed that furans showed a significant relationship with ageing, indicating that some components of this family could be possible markers of ageing.

1. Introduction

Cava is a sparkling wine (Certified Brand of Origin) produced using a traditional method in which a base wine is refermented in a sealed bottle. Wines have to be aged in contact with lees for a minimum period of 9 months in order to be considered Cava [1]. Also, Cava is classified in different categories according to the ageing time: Cava (9 months), Reserve (18 months) and Gran Reserve (30 months or more) [2]. During this ageing period yeast autolysis (lees) takes place, which implies the release of cell components and their breakdown products into the wine [3,4]. It is known that the contact between wine and lees improves the quality of the final product

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[5].

Together with colour and foam, aroma is one of the most important qualitative parameters of a wine. Volatile compounds in sparkling wines have three different origins: the grape, yeast metabolism during the two alcoholic fermentation steps (base wine and sparkling wine), and the ageing process while in contact with lees [6,7]. Studies on the behaviour of volatiles according to its ageing time have shown an increase in varietal compounds such as norisoprenoids, lactones and furans, and a decrease in acetate esters over time [8–10]. Thus, Cava loses its freshness and the characteristic fruity notes of the base wine and develops complexity with notes such as 'toasty', 'yeasty' and 'lactic' [5,10].

Also, changes in the volatile composition of wines occur during commercial storage [11,12]. Factors such as temperature, time, light exposure, type and position of the bottle, and dissolved oxygen influence the quality of the aroma [11,13–16]. The effect of temperature during storage has been widely studied, with an increase in some compounds such as furans while others such as terpenes decrease with storage temperature [16,17]. Various off-flavours such as methional and 1,1,6-trimethyl-l,2-dihydronaphthalene (TDN) may also increase due to changes in pH and temperature [18,19]. However, only a few studies have focused on the commercial storage of Cava sparkling wines and these centered on phenolic composition [20,21]. Thus, the behaviour of the aroma compounds during shelf life in Spanish sparkling wines is not well understood.

The aim of the current study was to assess the role of lees in the volatile profile of Cava (Spanish sparkling wine) by comparing two series of industrial Cava that were monitored during biological ageing and their corresponding series in commercial storage at the same temperature (16 °C).

2. Material and methods

2.1. Samples

A parallel study was designed to compare biological aging and commercial aging (Fig. 1). Sparkling wine was produced using the traditional method on an industrial scale at Freixenet S.A. winery (Sant Sadurní d'Anoia Barcelona, Spain). Bottles of 750 mL were bottled after the addition of the same "liqueur de tirage". This liqueur was mixed with the base wines, giving around 1–2 x 10^6 yeast cells mL^{-1} (yeast starter), 22–24 g of sucrose L^{-1} and 0.04 g L^{-1} per bottle of Calcium bentonite (Bentosan, acquired from Gon-Cruz S. L. La Rioja, Spain). The yeast employed was that of Freixenet S.A. wineries private collection. Afterwards, each Cava series was stored at 16 °C in the cellar for the second fermentation and ageing in contact with lees. Following the second fermentation, and after the 9 months in contact with lees (minimum legal time required to be considered cava), 40 bottles were sampled.

Half of the bottles (n = 20) were disgorged in order to remove the lees and brut expedition liquor was added to produce commercial sparkling wine. The remaining bottles (n = 20) were stored in contact with lees (biological aging) in the cellar at 16 °C. At this point, four bottles of commercial sparkling wine and four bottles of biological sparkling wine (point zero) were opened and analyzed. The same operation was performed at 4, 8, 12 and 21 months of aging. These aging times were considered to take in account the representative aging periods: 9 and 13 months (Cava), 17 and 21 (Reserve) and 30 (Gran Reserve).

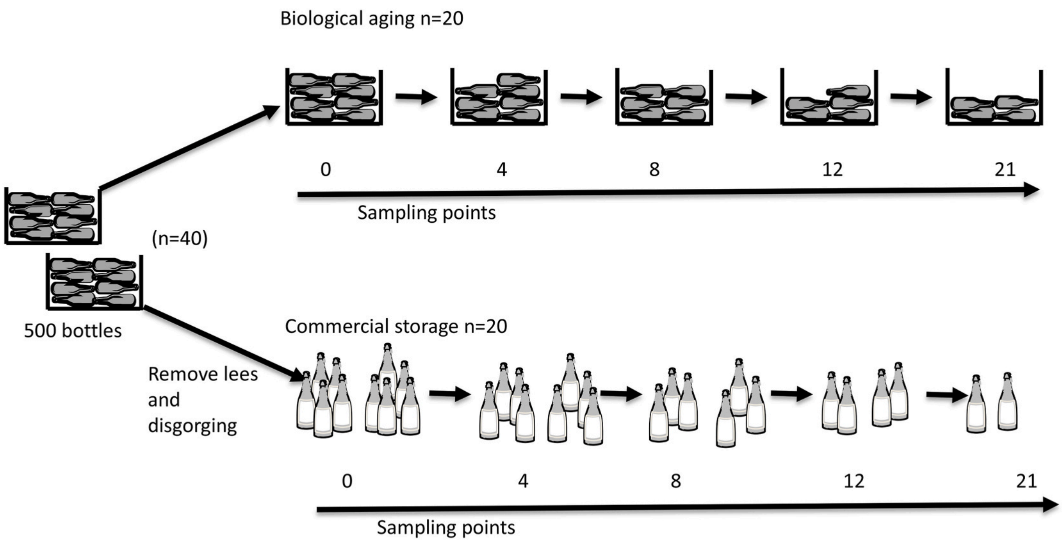

Fig. 1. Sampling scheme. A parallel study of the evolution of sparkling wines during biological aging and commercial storage. Sampling was performed at 0, 4, 8, 12 and 21 months, with four bottles of each sparkling wine sampled at each point for each type of aging (biological and commercial).

2.2. Chemicals and reagents

Furfural, ethyl isovalerate, hexanol, isoamyl acetate, γ-butyrolactone, benzaldehyde, hexanoic acid, ethyl hexanoate, limonene, ethyl 2-furoate, 1-octanol, 2-nonanone, 2-nonanol, 2-phenylethanol, diethyl succinate, ethyl octanoate, octanoic acid, β-cyclocitral, 2-phenylethyl acetate, nonanoic acid, ethyl nonanoate, decanoic acid, β-damascenone, ethyl decanoate, tetradecane, isoamyl octanoate, lilial, dodecanoic acid, ethyl dodecanoate, hexadecane, tetradecanoic acid, ethyl pentadecanoate, methyl pentadecanoate and ethyl hexadecanoate were purchased from Sigma-Aldrich-Fluka (St. Louis, Missouri, USA). Aliphatic hydrocarbons (retention index mixture for GC (KI) with purity above 95%) were also supplied by Sigma-Aldrich.

A 2 cm long Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) fibre was supplied by Supelco (Bellefonte, PA, USA). Before extraction the fibre was conditioned according to the manufacturer's recommendations.

2.3. Analysis of volatile composition

Extraction and quantification of the volatile compounds was performed using Headspace-Solid Phase Microextraction (HS-SPME) coupled to Gas Chromatography-Mass Spectrometry (GS-MS) according to Torrens et al. (2004) [22] methodology. Briefly, the fiber was activated each day before use by inserting it into the GC injector at 270 °C for 30 min. Two milliliters of sample were extracted over 40 min at 50 °C. The equilibration time was 20 min. An internal standard of 25 μL of nonanoic acid was added (0.1 mg/L).

Chromatographic analysis was carried out in a GC Focus coupled to a Mass Spectrometer Detector MS DSQII (Thermo Fischer Scientific, Waltham, MA, USA). Helium was used as a carrier gas. Separations were accomplished in a Factor Four Capillary column FV-5ms (30 m × 0.5 mm x 0.25 μm) (Agilent, Palo Alto, CA, USA). The injector temperature was set at 250 °C with a desorption time of 2.5 min. The initial temperature was 50 °C for 3 min, and this was subsequently increased at 3 °C/min using splitless injection mode for 5 min up to 260 °C for 5 min. GC-MS detection was performed in complete scanning mode (SCAN) in the 40–350 amu mass range with two scans per second. Electron impact mass spectra were recorded at an ionization voltage of 70 eV and ion source of 280 °C.

Compounds were identified by comparison of their mass spectra (MS) and their retention times (S) when pure standard was available. Moreover, identification was completed by comparison of the mass spectrum with the mass spectra library database Wiley 6.0. In addition, the Kovats Index (KI) was determined with reference to a homologous series of aliphatic hydrocarbons and compared with those available in the literature for the column FV-5ms (30 m × 0.5 mm x 0.25 μm). Quantification was achieved using the internal standard method. The volatile compounds identified were quantified by considering the relative response factor to be 1 and were expressed as mg/L equivalents of IS.

Odor Activity Values (OAV) were also calculated by dividing the concentration of each compound with their Odor Detection Threshold (ODT) referenced in the literature.

2.4. Statistical procedures

Principal components analysis (PCA) was performed to visualize the original arrangement of the Cava samples in an n-dimensional space by identifying the direction in which most of the information was retained and to show the separation between groups (biological and commercial storage). The first two principal components were used to plot the scores and loadings. Analysis of variance (ANOVA) was used to compare the differences between groups (with and without lees). The difference was considered significant when $p < 0.05$. Linear regression was used to determine the relationship between time and volatile compounds. Data were processed using Statgraphics centurion XV (Statistical graphics Co., Rockville, MD).

3. Results and discussion

The main objective of this study was to evaluate the role of yeast lees in the volatile profile of Cava (sparkling wine). Therefore, volatile compounds were monitored during the shelf life of sparkling wines over the same ageing period as in wines in contact with lees. It is well known that time and temperature influence the shelf life of a wine [14,17,23]. For this reason, temperature was maintained at cellar temperature (16 °C) to evaluate the role of lees and ageing time. When the amount of volatile compounds at 9 months of ageing in wine in contact with lees and without lees was compared, it was found that most of the compounds decreased after the disgorging stage (Table 1). This could be attributed to the removal of the lees. In fact, previous work by the research group showed the ability of the lees to retain aromas on their surface [24].

In order to study the behaviour of the volatiles during the ageing or shelf life of sparkling wines the evolution of some volatile profiles was evaluated. The volatiles assessed were some of those previously reported in the literature as a significant ageing markers or the main compounds determined in Cava profile: ethyl esters (e.g. ethyl hexanoate, ethyl octanoate, ethyl decanoate and ethyl dodecanoate, diethyl succinate), acetate esters (e.g. isoamyl acetate, phenylethyl acetate), furanes (furfural and ethyl furoate), alcohols (hexanol and 2-phenylethanol), norisoprenoids (e.g. TDN, vitispirane, β-damascenone, β-cyclocitral), fatty acids (hexanoic acid, octanoic acid, decanoic acid, dodecanoic acid) and limonene and benzaldehyde.

Table 1 lists the compounds tentatively identified in sparkling wines grouped according to the chemical family; the chemical name, the CAS number, the identification method used and the experimental Kovats indexes are also included. Table 1 also shows the amount of each volatile expressed in mg equivalent/L at the initial (after disgorging) and final ageing point (30 months) of the sparkling wine samples with (biological ageing) and without lees (commercial ageing). Also, in order to indicate how each compound might contribute to the aroma of sparkling wines Table 1 shows the odor note, if available in the literature. More than 60 volatile compounds

Table 1
List of the compounds detected in aged cava in contact with lees and stored cava, method of identification and initial (disgorging point) and final (30 months) levels.

	Compound	CAS	Identification	KI FV-5ms	Biological ageing		Commercial storage		odor note
					initial	final	initial	final	
	ethyl esters								
2	ethyl isovalerate	108-64-5	MS, KI, S	852	59	87	4	8	cashew, fruity, anise, sweet fruit, apple, blackcurrant[a]
9	ethyl hexanoate*	123-66-0	MS, KI, S	1003	4.903	5.233	0.860	0.501	apple peel, fruit[b]
12	ethyl 2-hexenoate	27829-72-7	MS, KI	1046	19	28	3	nd	fruity, slightly pungent[a]
21	diethyl succinate*	123-25-1	MS, KI, S	1184	1.282	2.081	1.422	0.629	wine, fruit[b]
22	ethyl octanoate*	106-32-1	MS, KI, S	1195	6.287	6.801	0.735	0.558	fruit, fat[b]
30	diethyl pentadienoate		MS	1283	4	14	1	5	
31	ethyl nonanoate	123-29-5	MS, KI, S	1296	32	7	4	0.000	fatty, fruity brandy-like[c]
39	ethyl decanoate	110-38-3	MS, KI, S	1396	126	60	12	16	grape[b]
52	ethyl dodecanoate	106-33-2	MS, KI, S	1595	11	14	2	4	leaf[b]
60	ethyl pentadecanoate	41114-00-5	MS, KI, S	1894	1	1	nd[e]	nd	
61	methyl pentadecanoate	7132-64-1	MS, KI, S	1907	2	1	nd	nd	
62	ethyl hexadecanoate (Ethyl palmitate)	628-97-7	MS, KI, S	1993	16	16	nd	nd	wax[b]
	acetate esters								
4	isoamyl acetate	123-92-2	MS, KI, S	878	166	69	17	5	banana[b]
27	2-phenylethyl acetate	101-97-3	MS, KI, S	1256	50	75	8	4	rose, floral, fruity, sweet[a]
	alcohols								
3	hexanol	111-27-3	MS, KI, S	869	77	89	9	33	resin, flower, green[b]
16	1-octanol	111-87-5	MS, KI, S	1074	31	45	1	2	chemical, metal, burnt[b]
18	2-nonanol	628-99-9	MS, KI, S	1105	25	9	1	nd	cucumber[b]
19	2- phenylethanol*	60-12-8	MS, KI, S	1115	1.449	1.249	0.137	0.423	honey, spice, rose, lilac[b]
	furans								
1	furfural	98-01-1	MS, KI, S	837	17	78	8	8	bread, almond, sweet[b]
13	ethyl 2-furoate	614-99-3	MS, KI, S	1053	29	40	nd	9	
46	furane derivative	–	MS	1477	12	26	1	7	
	acids								
8	hexanoic acid	142-62-1	MS, KI, S	996	15	36	11	61	sweat[a]
23	octanoic acid	124-07-2	MS, KI, S	1211	439	235	1	170	sweat, cheese[b]
28	nonanoic acid	112-05-0	MS, KI, S	1273	2	1	3	19	green, fat[b]
33	4-methoxyphenylacetic acid	104-01-8	MS	1313	0.000	1	1	4	
37	decanoic acid	334-48-5	MS, KI, S	1369	205	129	42	59	rancid, fat[b]
50	dodecanoic acid	143-07-7	MS, KI, S	1562	5	5	2	8	dry, metallic, weak, fatty, waxy odor[a]
58	tetradecanoic acid	544-63-8	MS, KI, S	1761	8	8	2	36	very faint, waxy-oily, nearly odorless[a]
	carbonyl compounds								
40	tetradecane	629-59-4	MS, KI, S	1400	2	5	nd	nd	alkane[b]
53	hexadecane	544-76-3	MS, KI, S	1600	6	9	nd	nd	alkane[b]
	terpenes, lactones, C^{13} norisoprenoids								
5	γ- butyrolactone	96-48-0	MS, KI, S	903	4	nd	1	5	caramel, sweet[b]

(*continued on next page*)

Table 1 (*continued*)

	Compound	CAS	Identification	KI FV-5ms	Biological ageing		Commercial storage		odor note
					initial	final	initial	final	
7	geranic oxide [d]	7392-19-0	MS	972	5	21	nd	nd	
10	limonene	5989-27-5	MS, KI, S	1030	170	32	2	62	citrus, mint[b]
11	*t*-β-ocimene (*m/z* 93, 91, 105, 121)	–	MS	1035	nd	nd	nd	76	citrus, herb, flower[b]
14	terpene (*m/z* 93, 121, 136)	–	MS	1059	nd	nd	nd	6	
20	nerol oxide	1786-08-9	MS, KI	1154	4	15	nd	1	oil, flower[b]
24	β-cyclocitral	432-25-7	MS, KI, S	1215	2	4	0.000	0.000	mint[b]
25	carene-unknown	13466-78-9	MS	1230	4	4	nd	nd	
29	2(1H)-naphthalenone-3,4,4a,5,6,7-hexahydro-1,1,4a-trimethyl	4668-61-5	MS	1279	239	463	0.002	17	green, fat, musty, sweaty, sour[a]
32	vitispirane	65416-59-3	MS, KI	1307	67	12	0.007	6	
36	1,1,6-trimethyl-1,2-dihydronaphthalene (TDN)	30364-38-6	MS, KI	1353	11	18	0.029	16	
38	β-damascenone	23726-91-2	MS, KI, S	1380	6	19	0.043	0.000	apple. Rose, honey[b]
41	1,4-methanoazulene, decahydro-4,8,8-trimethyl-9-methylene-longifolene	475-20-7	MS, KI	1408	4	6	0.002	9	
47	naphthalene, 1,2,3,4-tetrahydro-5,6,7,8-tetramethyl	19063-11-7	MS	1487	2	3	nd	3	
48	lilial	80-54-6	MS, KI, S	1526	6	4	0.003	nd	
49	terpene (*m/z* 93, 107, 122, 161)	–	MS	1534	9	9	nd	nd	
54	naphthalene derivative	–	MS	1632	3	4	nd	nd	
55	naphthalene derivative	–	MS	1637	0.000	1	nd	nd	
57	β-methylionone	127-43-5	MS	1658	5	6	nd	nd	
59	naphthalene derivative	–	MS	1839	1	3	0.009	4	
	aldehydes, ketones								
6	benzaldehyde	100-52-7	MS, KI, S	964	13	13	nd	4	almond, burnt sugar[b]
17	2-nonanone	821-55-6	MS, KI, S	1094	27	19	nd	nd	hot milk, soap, green[b]
35	2,4,4-trimethyl-3-(3-methylbutyl) cyclohexen-2-enone		MS	1343	5	2	nd	nd	
	other compounds								
15	isoamyl lactate	19329-89-6	MS	1069	12	12	nd	3	
26	isoamyl hexanoate	2198-61-0	MS, KI	1259	9	2	2	1	
34	unknown compound	–	MS	1331	4	5	nd	2	
42	2-propenal,3-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	4951-40-0	MS	1421	15	25	5	2	
43	unknown compound	–	MS	1430	5	21	4	nd	
44	isoamyl octanoate	2035-99-6	MS, KI, S	1441	nd	1	1	nd	
45	unknown compound	–	MS	1468	28	28	5	2	
51	Unknown propanoate compound (*m/z* 71, 111, 159, 243)	73381-40-1	MS	1586	4	4	2	nd	
56	isoamyl decanoate	2306-91-4	MS	1645	4	6	nd	nd	

Results are expressed as μg eq. L-1 except those marked with an asterisk (*), which are expressed as mg eq. L-1.
MS: mass spectra; S: pure standard; KI: Kovats index.
[a] http://www.flavornet.org/flavornet.html.
[b] http://www.pherobase.com.
[c] http://www.fao.org/ag/agn/jefca-additives/search.html?lang=es.
[d] Tentatively identified.
[e] Not detected.

were identified and quantified from several chemical families, including alcohols, esters, terpenes, and sesquiterpenes, C13-norisoprenoides, fatty acids, furfurals, aldehydes and lactones.

Quantitatively, the main components identified were ethyl esters such as ethyl hexanoate, ethyl octanoate and 2-phenylethanol. Total ethyl esters accounted for more than 70% of the volatile composition of sparkling wines (Table 1). These compounds come from the first and second fermentation and are responsible for the fruity note in sparkling wines [5,10,25]. Diethyl succinate was also identified; this compound was previously described as a post fermentative compound and was proposed as an age marker in aged Cavas [9,10]. Acetate esters were also identified in the Cava profile (isoamyl acetate, 2-phenylethyl acetate) and these are responsible for the fruity note in wines. The ethyl esters of medium molecular weight increased over time in the samples in contact with lees, while in Cavas without lees they tended to decrease (Table 1). This might be because lees tend to adsorb more easily such volatile compounds, thus decreasing their presence in the sparkling wine [10]. Moreover, it has been reported that the volatile profile of sparkling wine can depend on the retention and release of such compounds by lees during biological ageing [7,26]. Ethyl esters of high molecular weight showed a different behaviour. In commercial Cava the content of decanoate and dodecanoate increased during storage of the bottle. According to Makhotkina et al. (2012) [17], hydrolysis and esterification reactions are favoured at the pH of wine. However, other factors such as storage temperature and the initial concentration of fatty acids may have an influence on the behaviour of corresponding ethyl esters.

Minor compounds were also found in the volatile profile of sparkling wines, including alcohols, fatty acids and furan and naphthalene derivatives responsible for the sensory profile of aged sparkling wines (Table 1). Among the alcohols, hexanol, 2-phenylethanol, 1-octanol and 2-nonanol were tentatively identified. These compounds are responsible for the flower and/or vegetable notes of sparkling wines [10].

Fatty acids are responsible for the rancid and lactic notes of wines and have been described elsewhere [5,9,27]. They originate from the grape and, mainly, as a product of the metabolism of microorganisms during alcoholic fermentation in white winemaking conditions [28]. According to the chain length of fatty acids, they can more or less easily be absorbed on the cell wall of yeasts.

According to Makhotkina et al. (2012) [17], fatty acids are not affected by storage temperature. In the present study, all fatty acids increased over time in the commercial samples (without lees), probably due to the ester hydrolysis in wine. While the behaviour with lees was more variable, the content of octanoic, decanoic and nonanoic acid mainly decreased when in contact with lees while hexanoic acid increased. This is probably associated with the lactic note described previously in Gran Reserve Cava [5].

Furans are derived from sugar degradation and are responsible for the yeast and toasty notes of sparkling wines [5,29]. In the current study, furfural and ethyl 2-furoate were identified in the samples. Benzaldehyde is also produced in the Maillard reaction by Strecker degradation of amino acids or an alternative pathway of shikimic acid, with phenylalanine being an intermediate [29]. Furans increased during ageing with and without lees. The amount of furan compounds in wines was higher before disgorging, as was the case for other compounds.

C13-norisoprenoids were another chemical class tentatively identified in the volatile profile of sparkling wines, some of them for the first time. Some of these have previously been detected in the yeast lees surface [24] while others such as TDN, vitispirane and damascenone have been detected usually in the aroma of Cava. Norisoprenoids are varietal compounds derived from carotenoids such as β-carotene and lutein, or glycosidic precursors liberated during fermentation or ageing in contact with lees [30,31]. In the current study β-cyclocitral, vitispirane, TDN, β-damascenone, and some naphthalene derivatives were tentatively identified in the volatile profile of sparkling wine (Table 1). Norisoprenoids mainly appear in the largest quantities when wine is aged in contact with lees (Table 1). After the sparkling wine had been disgorged, compounds such as TDN, vitispirane, and damascenone decreased during storage. The characteristic structure of these compounds suggests that they could be important in the aroma of sparkling wine because they have a low olfactory perception threshold [31]. For example, β-damascenone is a ubiquitous compound with an extremely low odor threshold of 50 ng/L in hydroalcoholic solution [32] and is considered a positive contributor to wine aroma [33]. It has been described as having floral and exotic fruity notes. Norisoprenoids were previously identified in aged Port and aged Riesling wines [31,34] and Chilean sparkling wine [10]. In fact, Ubeda et al. (2019) [10] observed that some norisoprenoid compounds increased during ageing and propose the use of vitispirane as an age marker for young sparkling wines (less than 12 months ageing) rather than the usual TDN.

In addition, some terpenes including limonene, geranic oxide, t-β-ocimene, nerol oxide and lilial were tentatively identified (Table 1). These compounds are associated with floral and citric notes. They originate from grape skin or arise from grape precursor. The profile and behaviour of terpene compounds may be modified during ageing usually due to acid-catalyzed reactions. In general, the content of terpenes was higher in samples that had undergone biological ageing, but their individual behaviour was variable. Geranic and nerol oxide increased during biological ageing while lilial and limonene decreased. In commercial samples, limonene, ocimene, and nerol oxide increased while lilial still decreased (Table 1).

Long periods of contact between lees and wine during biological ageing (minimum 9 months) promote the interaction between lees cells and volatile compounds and permits the establishment of partition equilibriums [24,35]. In particular, the most hydrophobic volatile compounds can be adsorbed on the cell walls of lees [24], including esters, aldehydes, and terpenes [10,36], and thus their concentration in commercial sparkling wine must be reduced. Only some terpenes and norisoprenoids such as t-β-ocimene, TDN, and β-damascenone and some acids such as nonanoic, tetradecanoic and 4-methoxyphenylacetic acid, and ethyl pentadecanoate showed a higher concentration in commercial sparkling wine than in Cava aged in contact with lees. β-damascenone and TDN are the main norisoprenoids detected in surface lees [24]. In the current study, these compounds were found at higher levels in commercial Cava than in biologically aged Cava, showing that although they are more retained in lees surface, their presence in the volatile profile of Cava is significant. According to Gallardo-Chacon et al. (2009) [24], the high hydrophobicity of ester compounds explains their great retention on the surface of the lees. However, in the current study other compounds with less hydrophobicity such as phenylethanol or hexanol were retained in the cell walls of the lees, resulting in lower levels after the disgorging step.

In order to visualize the original arrangement of the Spanish sparkling wine in the space and show the separation of groups and the main components of each group a Principal Component Analysis (PCA) was performed (Fig. 2). The main differences were due to the type of ageing, i.e. with or without lees. In this sense, component 1 (44%) showed the differences between samples stored without lees,

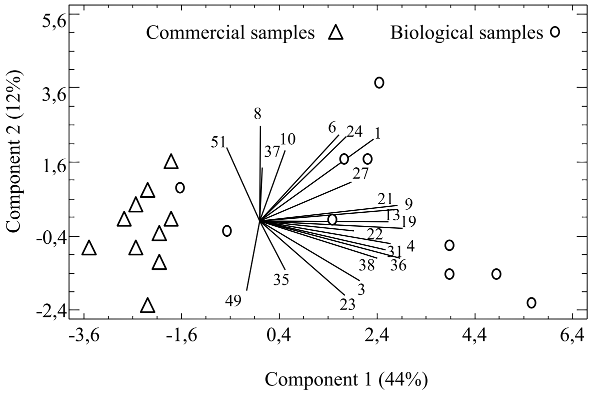

Fig. 2. Principal component analysis (PCA) of volatiles according to the ageing time and aged with and without lees. The numbers correspond to those in Table 1.

in the left of the plane while the samples stored in contact with lees could be found in the right (Fig. 2). Those samples aged in contact with lees had higher levels of the volatile compounds assessed. Analysis of variance revealed significant differences in the levels of some of the volatiles (furfural, hexanol, isoamyl acetate, ethyl hexanoate, ethyl furoate, phenylethanol, dietyl succinate, ethyl octanoate, cyclocitral, vitispirane, and decanoic acid) between the two types of ageing, being higher in all cases in samples aged in contact with lees ($p < 0.05$).

In addition, a multifactor analysis of variance was carried out considering the data of the sparkling wines studied (Table 2). It showed that the most significant variable affecting the amount of volatile compounds in sparkling wine was, in fact, the presence of yeast lees during ageing ($p < 0.05$). For example, diethyl succinate, that has been proposed as an ageing marker [9,10], was conditioned by the presence or absence of lees during ageing more than for the ageing period itself. The same happened with most of the ethyl esters found in the sparkling wines analyzed. Moreover, most of these compounds showed higher OAV in Cava aged in contact with lees, highlighting the importance of biological ageing over the ageing period.

Finally, in order to study the evolution over time of volatile compounds in biological and commercial samples a simple regression analysis was performed. Given that significant differences were observed between the two ageing types, the evolution of volatiles of Cava samples was assessed separately for each type (commercial and biological), with simple regression analysis revealing a relationship between ageing time and some furans (Fig. 3). Furans are compounds that are produced during the Maillard reaction by the dehydration of sugars [29]. According to Pereira et al. (2014) [28] furans are present at levels of less than 0.3% in dry wines; the

Table 2
Multifactor analysis of variance carried out considering the data of the sparkling wines studied.

	Aging time		Lees		ODT [a]	OAV [b]	
	F-ratio	*P*-value	F-ratio	*P*-value		*Biological ageing*	*Commercial storage*
Furfural	1.52	0.253	22.1	**< 0.001**	3000	0.03	<0.01
Hexanol	1.56	0.242	8.04	**0.014**	2500	0.04	0.01
Isoamyl acetate	2.02	0.151	20.67	**0.001**	2	**34.50**	**2.50**
Benzaldehyde	0.80	0.547	2.74	0.122	350	0.04	0.01
Hexanoic acid	0.12	0.740	0.12	0.167	3000	0.01	0.02
Ethyl hexanoate	1.33	0.310	62.38	**< 0.001**	1	**5,23**	0.50
Limonene	0.80	0.546	1.53	0.238	10	**3,20**	**6.20**
Ethyl 2-furoate	0.88	0.501	21.27	**0.001**	na	–	–
2-phenylethanol	0.13	0.967	14.63	**0.002**	750	<0.01	<0.01
Diethyl succinate	0.71	0.600	4.76	**0.048**	na	–	–
Ethyl octanoate	0.98	0.451	26.27	**0.001**	2	**3.40**	0.28
Octanoic acid	0.35	0.840	2.09	0.172	3000	0.08	0.06
b-cyclocitral	0.51	0.730	12.28	**0.004**	5	0.80	<0.01
2-phenylethyl acetate	0.61	0.660	3.79	0.073	650	0.12	0.01
Vitispirane	1.28	0.329	10.19	**0.007**	na	–	–
TDN	0.53	0.302	1.16	0.716	na	–	–
Decanoic acid	0.39	0.813	19.00	**0.001**	10000	0.01	0.01
b-damascenone	0.47	0.760	0.27	0.613	0,002	**9500**	<0.01
Ethyl decanoate	0.78	0.557	4.70	**0.049**	na	–	–
Dodecanoic acid	0.57	0.689	0.84	0.377	10000	<0.01	<0.01
Ethyl dodecanoate	1.46	0.272	1.89	0.192	na	–	–

Na: not available.
[a] ODT: Odor Detection Threshold [37].
[b] OAV: Odor Activity Value, calculated by dividing the concentration of each compound with their ODT.

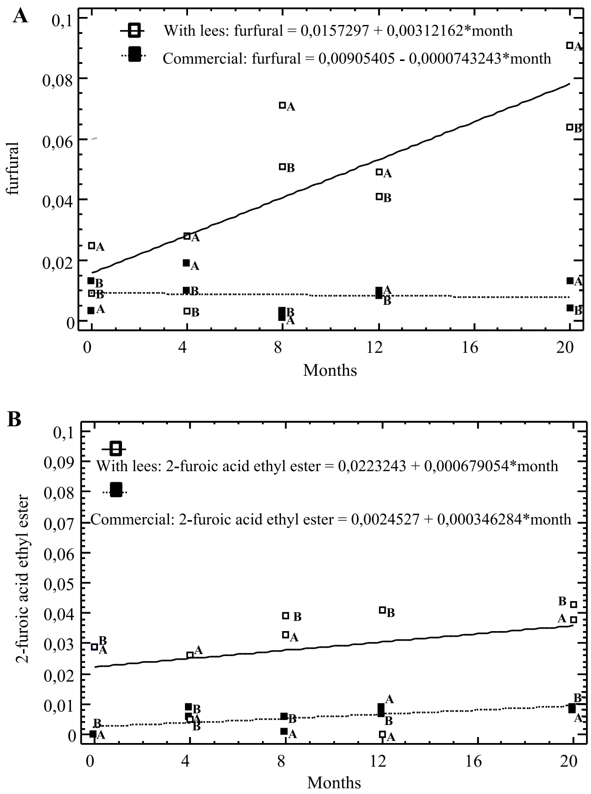

Fig. 3. Kinetics of Furfural (A) in Spanish sparkling wine aged with lees and Ethyl 2- furoate (B) stored without lees over time.

formation of these compounds was increased due to the Maillard reaction or degradation of sugars. Some previous studies found a correlation between the content of some furans and temperature. These compounds have been described previously in Spanish sparkling wines as producing bread, toasty, balsamic and caramel notes [5]. Torrens et al. (2010) [5] also detected more furans in Cava than in base wine and associated them with toasty notes in aged Cava. In the present study, furfural was associated with ageing time in biological ageing (in contact with lees) ($R2 > 0.7$) (Fig. 3A) while during commercial storage (without lees), ethyl 2-furoate was associated with ageing time ($R2 > 0.66$) (Fig. 3B). According to Makhtokina et al. (2012) [17], branched chain fatty acids such as ethyl 2-furoate could be formed during time in the bottle as the esterification reaction is favoured at the pH of wine. These preliminary results suggest an important role of the furans during ageing of Spanish sparkling wine whether in the presence of yeast lees or not, which may involve the sensory quality of wine.

4. Conclusions

In conclusion, more than 60 volatile compounds were tentatively identified in biologically aged Cavas, and these decreased in quantity during the disgorging step. The more characteristic compounds were evaluated over time, and it could be suggested that furan compounds play an important part in the volatile composition during ageing regarless of lees presence. However, more information about this family is needed in order to clarify the role of these compounds in the quality of aged Cavas.

Author contribution statement

Alba Martín-Garcia: Analyzed and interpreted the data; Wrote the paper. Clara Abarca-Rivas: Analyzed and interpreted the data. Montserrat Riu-Aumatell: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper. Elvira López-Tamames: Conceived and designed the experiments; Analyzed and interpreted the data.

Data availability statement

Data included in article/supp. Material/referenced in article.

Declaration of competing interest

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

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