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Using an electronic nose and volatilome analysis to differentiate sparkling wines obtained under different conditions of temperature, ageing time and yeast formats

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Decanoic acid (PubChem CID: 2969)
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Dihydrofuran-2(3H)-one (PubChem: 7302)
γ-Decalactone1 (PubChem CID: 12813)
1,3-Dimethoxy-2-hydroxybenzene (PubChem CID: 7041)
Ethyl acetate (PubChem CID: 8857)
Ethyl lactate (PubChem CID: 7344)
2-Ethylhexan-1-ol (PubChem CID: 7720)
Ethyl propanoate (PubChem CID: 7749)
Ethyl isobutanoate (PubChem CID: 7342)
Ethyl butanoate (PubChem CID: 7762)
Ethyl furoate (PubChem CID: 11980)
Ethyl hexanoate (PubChem CID: 31265)
Ethyl octanoate (PubChem CID: 7799)
Ethyl decanoate (PubChem CID: 8048)
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ABSTRACT

Effect of yeast inoculation format (F), temperature (T), and "on lees" ageing time (t) factors were evaluated on the composition of sparkling wines by a quantitative fingerprint obtained from volatile metabolites and the response of an electronic nose (E-nose). Wines elaborated according the traditional method at 10 and 14 °C, free cells and yeast biocapsules formats were monitored at 15 and 24 months of ageing time. Sixty-six volatiles identified and quantified in the eight sampling lots were subjected to a pattern recognition technique. A dual criterion based on univariate (ANOVA) and multivariate analysis (PLS-DA) through the variable importance projection (VIP) values, allowed to identify ten volatiles as potential markers for T factor, eleven for t and twelve for F factors. The discriminant models based on E-nose dataset enable a 100% correct classification of samples, in relation with t and F factors and the 83% for T factor.

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4-Ethenyl-2-methoxyphenol (PubChem CID: 332)
5-H-furan-2-one (PubChem CID: 10341)
(Furan-2-yl)methanol (PubChem CID: 7361)
Furan-2-carbaldehyde (PubChem CID: 7362)
5-(Hydroxymethyl-2-furaldehyde (PubChem CID: 237332)
Guaiacol (PubChem CID: 460)
Hexan-1-ol (PubChem CID: 8103)
Hexanoic acid (PubChem CID: 8892)
Hexyl acetate (PubChem CID: 8908)
Methanol (PubChem CID: 137654)
Methyl acetate (PubChem CID: 6584)
Isoamyl acetate (PubChem CID: 31276)
Isobutyl acetate (PubChem CID: 8038)
2-Methyl-propan-1-ol (PubChem CID: 6560)
2-Methyl-1-Butanol (PubChem CID: 8723)
3-Methyl-1-Butanol (PubChem CID: 31260)
Nerolidol (PubChem CID: 5284507)
Nonanal (PubChem CID: 31289)
Octanoic acid (PubChem CID: 379)
2-Oxepanone (PubChem CID: 10401)
Propan-1-ol (PubChem CID: 1031)
Pentanoic acid methyl ester (PubChem CID: 12206)
5-Pentyloxolan-2-one (PubChem CID: 7710)
2-Phenylethan-1-ol (PubChem CID: 7409)
2-Phenylethyl acetate (PubChem CID: 7654)
Trans-2-hexen-1-ol (PubChem CID: 5318042)
Tetradecanoic acid (PubChem CID: 11005)
Vitispirane (PubChem CID: 6450832)

1. Introduction

The traditional sparkling wine-making method (*Champenoise method*), is based on the so-called 'prise de mousse' stage, within which two steps can be differentiated: a second fermentation of a base wine and a subsequent minimum ageing of 9 months in contact with yeast lees in the same closed bottle (Torresi, Frangipane, & Anelli, 2011). Both steps lead to important changes in the organoleptic profile of sparkling wines, especially the ageing period where the yeast autolysis takes place (Alexandre & Guilloux-Benatier, 2006). This step has been widely studied because of the influence that the enzymes and products released from yeasts have on the foam properties and the final odorant profile (Kemp, Alexandre, Robillard, & Marchal, 2015). Once the ageing time is brought to an end, the product undergoes different stages that make it to acquire the ideal characteristics for its commercialization. Among them, highlight the stage known as 'riddling', whose purpose is to produce a coarse sediment facilitating the yeast's lees removal. Riddling stage entails an intensive and time-consuming workforce whose improvement awakens the interest of winemakers. However, the developments in the use of immobilized yeast systems may shorten both the time and the expenses of this process. (Moreno-Garcia, Garcia-Martinez, Mauricio, & Moreno, 2018). Immobilization systems involve the confinement of yeast cells in an external physical support meeting the food quality and safety standards. In this regard, the calcium alginate supports are widely recommended to sparkling wine-making due to their easy preparation and high degree of yeast adsorption (Busova, Magyar, & Janky, 1994). Nevertheless, some studies have shown that wines obtained from them had an increase in calcium and sodium ions that could alter their organoleptic properties (Lopez de Lerma et al., 2018; Puig-Pujol et al., 2013). Consequently, new immobilization systems, free of external supports or chemical binders, have been assayed, such as the so-called yeast biocapsules. These systems consist of a co-immobilization of *Saccharomyces cerevisiae* in filamentous fungi with GRAS status (such as *Penicillium chrysogenum*). Studies focused on applying biocapsules to the sparkling wine elaboration demonstrated that this immobilization system do not affects negatively their aroma quality (Puig-Pujol et al., 2013; Lopez de Lerma et al., 2018), showing some interesting applications for the oenology field (Ogawa, Bisson, Garcia-Martinez, Mauricio, & Moreno-Garcia, 2019).

It is widely recognized that sparkling wines of the highest quality are obtained by the traditional method and this quality increases according to the ageing time on lees. In this context, the aroma is considered a crucial indicator to assess their quality, with sensory evaluation by trained experts being one of the most important techniques. However, the formation of a sensory panel is not always available because it is expensive and time consuming. On the other hand, chemical analysis allows us to know, accurately and objectively, the key volatile compounds playing an important role in the sparkling wine aroma. Thus, some authors have used them as potential markers to ageing time monitorization, to avoid frauds (Fedrizzi, Magno, Finato, & Versini, 2010; Pozo-Bayon, Pueyo, Martin-Alvarez, Martinez-Rodriguez, & Polo, 2003; Riu-Aumatell, Bosch-Fuste, Lopez-Tamames, & Buxaderas, 2006; Ruiz-Moreno et al., 2017; Torrens, Riu-Aumatell, Vichi, Lopez-Tamames, & Buxaderas, 2010), and to the characterisation of the second fermentation (Martinez-Garcia, Garcia-Martinez, Puig-Pujol, Mauricio, & Moreno, 2017; Martinez-Garcia et al., 2020; Munoz-Redondo et al., 2017).

Over the last decades, new technologies are proposed to provide a global information related to the simultaneous detection of a high number of compounds. These holistic methodologies have been developed in combination with pattern recognition techniques to obtain a unique pattern or 'fingerprint', allowing to stablish a discrimination or classification procedure for samples of the same food type of different quality categories. Thus, the most popular method, applied to the assessment of wine aroma quality, is an electronic nose (E-nose), which is based on a multiple sensor array system (Rodriguez-Mendez et al., 2016). It is also well known that, to fulfil consumer preferences, the markets require highly standardised products in which batch to batch variations are not acceptable. Thereby, the use of E-nose is lately focused on the early detection of non-compliant batches to save the production time through the different stages involved (Sanaeifar, ZakiDizaji, Jafari, & de la Guardia, 2017). Regarding the wine-making, the most recent applications are focused on the different stages of still wines production, from grapes to the bottled products. (Buratti et al.,

2011; Lopez de Lerma, Bellincontro, Garcia-Martinez, Mencarelli, & Moreno, 2013; Lozano, Arroyo, Santos, Cabellos, & Horrillo, 2008; Lozano et al., 2015; Santonico, Bellincontro, De Santis, Di Natale, & Mencarelli, 2010; Wei, Yang, Liang, & Li, 2014). Among the different E-nose types proposed, the capabilities of those based on metalloporphyrins coated quartz microbalances have been demonstrated in different applications. Notwithstanding, less attention has been paid to their applications on the quality control of sparkling wines. (Franceschi, Tiranno, Vincenzi, Boatto, & Bravi, 2015).

Against this background, there are few studies related to the effect of yeast biocapsules system on the sparkling wine volatilome, although some are focussed on a specific ageing time. Therefore, comparison of changes during on lees ageing at different temperatures has not yet been possible (Puig-Pujol et al., 2013; Lopez de Lerma et al., 2018). Beyond that, to the best of our knowledge, there are no studies that compare the effects of these factors by using E-nose or by targeted volatilome for the quality control and differentiation of sparkling wines.

This work aims to complete previous studies based on the use of 'yeast-biocapsules' to elaborate sparkling wines under different conditions of temperature and ageing time. For this, the composition in wine volatiles and the potential of an E-nose is evaluated to discriminate among the wines obtained. This will allow to establish an easy and fast quality control procedure that may contribute to the prevention of frauds and adulterations.

2. Material and methods

2.1. Chemical standards

Standard solutions of analytical grade pure compounds from Sigma-Aldrich, Merck and Fluka were used for the identification and quantification of volatile compounds. Pure water was obtained from a Milli-Q purification system (Millipore).

2.2. Yeast strain, filamentous fungus and experimental design

Saccharomyces cerevisiae P29 strain (CECT 11770), was used as starter culture for the second fermentation in free and immobilized formats. This yeast was isolated in the Penedés grape-growing area (Spain) by the INCAVI (Institut Català de la Vinya i el Vi) and selected by their ability for sparkling wine production.

The filamentous fungus used for cell immobilization was *Penicillium chrysogenum strain H3* from the Department of Microbiology (University of Cordoba, Spain) collection.

2.2.1. Yeast starter cultures, acclimation and biocapsules formation

A volume of 45 mL of pasteurized must was inoculated with a pure culture of P29 strain from agar YEPD broth and was incubated at 21 °C for 48 h. The yeasts acclimation to the conditions imposed by the second fermentation was achieved following the usual INCAVI protocol, previously described by Martinez-Garcia et al. (2020). The pasteurized must was a blend of 50% Macabeo, 30% Xarel-lo, 20% Parellada grapes with the following characteristics: 178 g/L of reducing sugars, pH 3.4 and total acidity 3.6 g/L.

The formation of P 29 yeast biocapsules was reached by following the patented method by authors (WO 2012/175774 Al) and applied to sparkling wines by Puig-Pujol et al. (2013). Briefly, this method consists in a spontaneous co-immobilization between *P. chrysogenum* fungus and the yeast in a culture broth buffered to a pH = 7, containing yeast nitrogen base (YNB) and gluconic acid as only carbon source. Yeast cells counts in free and biocapsules format were carried out according to Lopez de Lerma et al. (2018), Martinez-Garcia et al. (2020) and added to the bottles to reach a cell population of 1.5 10^6 cell/mL of base wine in each format.

2.2.2. Base wine and fermentation conditions

The 'Cava' sparkling wine is a Certified Brand of Spanish Origin that is subjected to a strict elaboration normative. Thus, the base wine (BW) was obtained by a first alcoholic fermentation of a blend of *Vitis vinifera* (50% Macabeo, 30% Xarel-lo and 20% Parellada) varieties from the Penedés grape-growing area, (NE, Spain). This BW having an ethanol content of 10.8% v/v, a total acidity 6.4 g/L (as tartaric acid), a total SO_2 content of 90 mg/L and a pH value of 3.05, was added with 22 g/L sucrose and transferred to 50 sparkling wine bottles of 750 mL volume for the "foaming process". The bottles were divided in two batches, according to the two inoculation formats tested. The first batch was added with free cells (FC) and the second with yeast biocapsules (BIO), reaching in both cases a yeast population of 1.5 10^6 cells/mL and then, all the bottles were closed with a plastic lid and a crown cap. To study the temperature effect, bottles from each format were divided in two lots to carried out the second fermentation and the ageing on lees at 10 °C and 14 °C (10 T and 14 T, respectively), in conditioned chambers to each temperature. The second fermentation was followed by changes in the endogenous pressure of CO_2, measured with an internal aphrometer (Oenotilus, Station Oenotechnique de Champagne, Epernay, France) and was considered as finished when it reached 6 bar, which correspond to the total uptake of sucrose added. The effect of ageing time was followed for each temperature by taking samples at 15 and 24 months (15 M and 24 M). Three bottles of each condition were taken to perform the triplicate analysis (Supplementary Fig. 1).

2.3. Oenological parameters

The International Methods of Analysis of Wines and Musts (OIV, 2020) were used as reference methods of the parameters described below. Common oenological variables such as total acidity, volatile acidity, pH and ethanol content (% v/v), were analysed by Infrared Spectroscopy (FT-IR) in a Winescan 120 FOSS, (Rellingen Germany). Sugar, malic acid, lactic acid, ammonium, free amine nitrogen and yeast available nitrogen (YAN) were measured by enzymatic method using a multi-parametric analyzer Lisa 200 (Hycel Diagnostics, Technology Diffusion Iberica, Barcelona, Spain). Flame atomic absorption spectrometry was used to analyse calcium and potassium ions in a Perkin Elmer 280 (Madrid, Spain) device. The Total Phenol Index (TPI) considered as A_{280}, A_{320}, the chromatic parameters A_{420}, A_{520}, A_{620} and the CIELab space coordinates were evaluated in a UV–Vis spectrophotometer Lambda 25 (Perkin –Elmer, Massachusetts, USA). For the measurement of Nephelometric Turbidity Units (NTU) a turbidimeter Hach (Colo, USA) was used. Finally, the foam characteristics (maximum foam height (HM) and the plate height (HS) or foam persistence) were evaluated using the Mosalux procedure, following the protocol previously described by Martinez-Garcia et al. (2020).

2.4. Volatilome analysis

The wine volatilome is the sum of several hundred of volatile metabolites that are grouped according to their content in majority (contents ≥10 mg/L) and minority volatile compounds (< 10 mg/L) (Martinez-Garcia et al., 2017).

2.4.1. Majority volatiles and polyols

This fraction was determined in an Agilent 6890 Gas-Chromatograph (Palo Alto, CA) provided with a CP-WAX 57 CB capillary column (60 m, 0.25 mm i.d., 0.4 μm film thickness) from Varian (Palo Alto, CA), and equipped with a Flame Ionization Detector (FID). The chromatographic conditions and quantification procedure were described by Martínez-García et al. (2017, 2020). A volume 1 μL of wine sample spiked with an internal standard solution of 4-methyl-2-pentanol at 1 g/L, was injected in the inlet. An Agilent 7890A GC, coupled to a MSD5975C Mass Detector from Agilent Technologies (Wilmington, DE, USA) was also used to confirm the identification of

each compound.

2.4.2. Minority volatiles

This fraction of volatiles must be concentrated before analysis, because of their low content. Thus, a Stir-Bar-Sorptive- Extraction (SBSE) technique was used as liquid–solid extraction, following the protocol described by Martinez-Garcia et al. (2020). For this, a Stir Bar 'Twister' (0.5 mm film thickness and 10 mm length, from Gerstel GmbH, Mülheim an der Rühr, Germany) coated with polydimethylsiloxane (PDMS) was submerged in a 10 mL vial containing 1 mL sample and 9 mL buffer solution pH = 3,5, stirring at 1200 rpm for 100 min at 20 °C and then transferred into a desorption tube for GC–MS analysis.

The analytical platform consisted of: a multipurpose sampler (MPS), and a Thermal Desorption Unity (TDU) from Gerstel coupled to and Agilent 7890A- GC MSD 5975 system. The GC was fitted with an HP-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) and the operating conditions for GC and MSD were described by Lopez de Lerma et al. (2018). A detailed information about the quantification and identification criteria, is in supplementary material.

2.4.3. Calculation of odorant series

Each volatile compound contributes qualitatively to the overall wine aroma by their specific smell, which may be defined by one or several odorant descriptors. In addition, the quantitative contribution of a specific volatile can be estimated by its odour activity value (OAV), calculated by dividing the concentration of the compound by its odour perception threshold (OPT) (Lopez de Lerma et al., 2018). This "absolute" value constitutes a first approach because an "effective" value, calculated through the sensory interactions among the wine odorants, is revealed un-affordable so far. Considering this, the OAVs calculated for compounds with the same or similar descriptors can be added to constitute an odorant series (OS). In this study, OAVs were calculated for all the quantified compounds, being excluded those without pure compounds commercially available or without OPT available in the wine literature.

2.5. Electronic nose

The E-nose consisted of an array of eight quartz microbalances (QMB) coated with modified metallo-porphyrins (5,10,15,20-tetraphenylporphyrin, TPP), whose selectivity depends on the nature of both the central metal and the peripheral substituent of macrocycles (Mn-TPP, Co-*p*-OCH_3 TPP, Sn-TPP, Rh-TPP, Co-*p*-NO_2, Cr-TPP, Co-TPP, Ru-TPP). The QMB sensors act as an electromechanical resonator with a natural oscillation frequency ΔF of 20 MHz that change when volatiles interact reversibly with its surface. The signal from the sensors was acquired and formatted through software before processing and analysing it. A detailed description was previously made by Di Natale, Paolesse, and D'Amico (2007), and Santonico et al. (2010).

To carry out this analysis, the CO_2 gas of 50 mL wine sample was previously removed by partial vacuum using a 500 mL Erlenmeyer flask and a Venturi pump system for 1 min. Then, 5 mL of this sample were placed in a 10 mL vial and tempered at 25 ± 2 °C for 30 min. The volatiles in the vial headspace were then transferred into the E-nose sensor cells using an air stream during 10 min, which was filtered through a trap filled with anhydrous $CaCl_2$. Pure nitrogen (99.9998%) at a constant flow of 200 mL/min was used during 5 min to clean the sensors and the obtained signals were considered as reference for each

Table 1
Average values and (SD‡) of sparkling wine oenological parameters grouped according to temperature (T), ageing time (t) and format (F) factors and their interaction (TxtxF) effect.

	Temperature (T, °C)			Ageing Time (t, months)			Format (F)			Interaction
Parameter	10 T	14 T	p-Value	15 M	24 M	p-Value	FC	BIO	p-Value	p-Value
Ethanol (% v/v)	12.01(0.04)	12.0(0.1)	ns	12.03(0.03) b	11.97(0.03) a	***	12(0.04)	11.99(0.04)	ns	ns
Reducing sugars (g/L)	0.22(0.04)	0.24(0.05)	ns	0.3(0.1) b	0.2(0.0) a	***	0.22(0.04)	0.24(0.05)	ns	ns
Glycerol (g/L)	5.1(0.8) b	4.1(0.6) a	**	4.9(0.8)	4.4(0.9)	ns	4.4(0.7)	4.9(0.9)	ns	ns
Volatile acidity (g/L)	0.23(0.02)	0.23(0.02)	ns	0.25(0.01) b	0.21(0.01) a	***	0.23(0.02)	0.23(0.02)	ns	ns
Total acidity (g/L)	5.6(0.2)	5.5(0.1)	ns	5.6(0.1)	5.5(0.2)	ns	5.6(0.1) b	5.4(0.1) a	***	ns
pH	3.12(0.01)	3.13(0.01)	ns	3.12(0.01) a	3.13(0.01) b	*	3.12(0.01)	3.13(0.01)	ns	ns
Malic acid (g/L)	1.5(0.3)	1.5(0.3)	ns	1.8(0.1) b	1.28(0.04) a	***	1.5(0.3)	1.5(0.3)	ns	ns
Lactic acid (g/L)	0.4(0.1)	0.4(0.1)	ns	0.42(0.04)	0.40(0.08)	ns	0.43(0.05) b	0.37(0.07) a	*	**
TPI (AU 280 nm)	4(2)	4(2)	ns	5.9(0.1) b	1.24(0.03) a	***	4(2)	4(2)	ns	ns
A320 (AU 320 nm)	2(1)	2(1)	ns	3.4(0.1) b	0.71(0.02) a	***	2(1)	2(1)	ns	ns
A420 (AU 420 nm)	0.07(0.01) a	0.080(0.003) b	**	0.076(0.004)	0.08(0.01)	ns	0.08(0.01)	0.078(0.004)	ns	*
A520 (AU 520 nm)	0.008(0.001) a	0.011(0.002) b	***	0.009(0.002) a	0.011(0.003) b	*	0.010(0.003)	0.010(0.002)	ns	ns
A620 (AU 620 nm)	0.001(0.003)	0.001(0.001)	ns	0.0001(0.000) a	0.002(0.003) b	***	0.002(0.002)	0.001(0.001)	ns	ns
Color intensity (A420 + A520 + A620)	0.08(0.01) a	0.09(0.01) b	***	0.08(0.01) a	0.09(0.01) b	**	0.09(0.01)	0.09(0.01)	ns	ns
Tonality (A420/A520)	9(1) b	7(1) a	***	9(1)b	8(1)a	*	8(2)	8(1)	ns	ns
a*	0.02	0.23	ns	1.2(0.1) b	−0.9(0.6) a	***	0.1(1.2)	0.2(1.2)	ns	ns
b*	5.6(0.5) a	5.9(0.2) b	*	5.5(0.3) a	6.0(0.4) b	**	5.6(0.3)	5.8(0.5)	ns	ns
L*	99.5(0.2) b	99.3(0.3) a	*	99.6(0.1) b	99.2(0.2) a	***	99.4(0.3)	99.4(0.3)	ns	ns
C*	5.7(0.5) a	5.9(0.2) b	*	5.6(0.3) a	6.0(0.4) b	**	6.0(0.3)	6.0(0.5)	ns	ns
H*	102(1) b	101(1) a	*	102(1) b	100(1) a	***	101(1)	101(1)	ns	ns
HM (Maximum height, mm)	22(5)	21(5)	ns	22(6)	21(3)	ns	24(4) b	19(4) a	**	ns
HS (High stability, mm)	16(6)	12(6)	ns	12(6)	15(6)	ns	16(6)	12(5)	ns	ns
YAN (mg/L)	64(2)	65(6)	ns	65(2)	64(3)	ns	64(2)	65(3)	ns	ns
N amine (mg/L)	56(4)	57(3)	ns	60(2) b	53(2) a	***	56(4)	57(4)	ns	ns
N ammoniacal (mg/L)	8(3)	8(4)	ns	5(1) a	11(1) b	***	8(3)	8(3)	ns	*
NTU	1.5(1.4) a	5(4) b	*	3(2)	2(3)	ns	1(1) a	4(3) b	**	ns
Ca (mg/L)	89(8) a	95(2) b	*	95(4)	90(8)	ns	90(8)	95(2)	ns	***
K(mg/L)	299(107)	298 (106)	ns	197(0) a	400(4) b	***	298(106)	299(107)	ns	ns

10 T: sparkling wines elaborated at 10 °C. 14 T: sparkling wines elaborated at 14 °C. 15 M: sparkling wines with 15 months of ageing. 24 M: sparkling wines with 24 months of ageing. FC: sparkling wines elaborated with free cells. BIO: sparkling wines elaborated with bio-immobilized cells. Significant level: ns = non-significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. n.f. = not found.. Letters a, b, L, C and H correspond to the Cielab color coordinates. HM and HS are parameters used for foam properties. TPI: Total Phenol Index. YAN: Yeast Assimilable Nitrogen. NTU: Nephelometric Turbidity Unit. . ‡ standard deviation calculated by grouping the samples for which the analysed factor has the same value.

QMB. For each wine sample two vials were prepared in order to analyse their headspace by E-nose and the mean values of each QMB were used for statistical treatment.

2.6. Statistical analysis

All data showed in tables are the average values of three biological replicates, analysed in triplicate for each studied condition and were previously pre-processed, by autoscaling and centering. Univariate analysis (ANOVA) was performed through Statgraphics Plus v. 2, de STSC, Inc. (Rockville, MD, USA) software. Oenological parameters and volatile compounds were processed using two-way analysis of variance and LSD Fisher s test to study the individual effect of temperature, time and yeast format used (free or biocapsules) and their interaction through multivariate analysis of variance (MANOVA). Differences at $p \leq 0.05$ were considered significant. Also, homogeneous groups analysis (HG) and multiple variable analysis (MVA) were carried out to get a fingerprint of the wine analysed.

The cross-platform integrated development environment for the statistical language (Rstudio) was used to perform unsupervised pattern recognition techniques such as Heatmap and Principal Component Analysis (PCA). These techniques allow to establish differences among wine samples in relation to the factors studied and the methodology used for analysis (GC or E-nose). Finally, supervised recognition techniques such as partial least square discriminant analysis (PLS-DA) were also applied to study the E-nose discriminant power and to select potential volatile markers through the variable importance projection (VIP) values.

3. Results and discussion

3.1. Development of the second fermentation

The processes carried out at 10 °C showed slower CO_2 production kinetics than the fermented ones at 14 °C. Yeasts inoculated in free format began the fermentation before yeast biocapsules at 14 °C (day 4 and 13, respectively) and also at 10 °C (day 11 and 25, respectively). This delay is mainly a consequence of the yeast stress (Puig-Pujol et al., 2013) because the micro-structure of the biocapsules reveals that yeasts are entrapped in the hyphae net, allowing to a good mass transfer (Ogawa et al., 2019). Moreover, the interactions between base wine, temperature and yeast strain have a strong effect on the fermentation kinetics (Marti-Raga, Sancho, Guillamon, Mas, & Beltran, 2015). Anyway, the two inoculum formats successfully completed fermentation at 60 and at 109 days (14 and 10 °C, respectively), reaching between 6 and 7 bars of pressure in both cases.

3.2. Oenological features

Table 1 shows the average values and standard deviation for the 24 wine samples and the results of two- way ANOVA carried out to stablish the significant homogeneous groups (HG) at $p \leq 0.05$ level for the two values considered in: temperature (*T*), time (*t*) and format (*F*) and their interaction (*TxtxF*). According to the obtained results, none of the oenological variables are dependent on the three factors analysed, although the contents in lactic acid, A_{420}, N ammoniacal and Ca, are affected by their interaction. Chromatic parameters and CIELab coordinates showed significant relationships with temperature and ageing, being the wines obtained at 14 °C and 24 months characterized with higher values in color intensity (CI), tonality, b*, C* and lower values in the parameters a*, L* and H*. Changes in chromatic characteristics were also found during the winemaking and ageing by (Torchio, Segade, Gerbi, Cagnasso, & Rolle, 2011; Torrens et al., 2010). Furthermore, wines obtained at 14 °C showed higher values for calcium content and turbidity but lower values in glycerol, although higher than those reported for young 'cavas' at 14 °C (Puig-Pujol et al., 2013). No changes in foam parameters (HM and HS) were observed due to the temperature effect, unlike what was described by Esteruelas et al. (2015). On the other hand, "on lees" ageing time affected a higher number of parameters. Thus, wines aged for 24 months reached higher values in pH, N ammoniacal and potassium contents and lower in ethanol, reducing sugars, malic acid, N amine, volatile acidity, A_{280} and A_{320}. Related to these parameters, no changes were described in literature when trying to compare 'cavas' 14 months aged with 24 months aged (Torrens et al., 2010). Finally, yeast format only affected significantly the total acidity, lactic acid, the foam parameter HM and turbidity. Despite the differences found, all the obtained wines fulfilled the legal and quality standards established in the Spanish normative (BOE, 2020).

3.3. Wine volatilome

Supplementary Table 1 shows the CAS number, LRI values (NIST, 2020), odour descriptors, perception threshold (OPT) and the odorant series (OS) for 67 metabolites identified in this work. All the compounds are considered wine volatiles, exception made for the polyols 2,3-butanediol and glycerol, which are semi-volatile and non-volatile compounds, respectively. Ten volatiles are classified as major and the remaining as minority aroma compounds. According their chemical structure there are identified: 9 alcohols, 6 aldehydes, 3 ketones, 5 carboxylic acids, 24 esters, 5 lactones, 1 terpenoid, 2 norisoprenoids, 5 volatile phenols, 4 furanic compounds and 2 polyols. To stablish comparison with other authors, esters were also grouped according to Ruiz-Moreno et al. (2017) as ethyl ester of fatty acids (EEFAs), ethyl esters of branched acids (EEBAs), methyl esters of fatty acids (MEFAs), isoamyl esters of fatty acids (IEFAs) and higher alcohols acetates (HAAs). The criteria used for the identification of volatiles has been described in the Supplementary material.

Regarding to the contents in wines of chemical families (Supplementary Table 2), the alcohols have the highest average values (from 290 to 441 mg/L), followed by EEFA (91–129 mg/L), aldehydes (54–89 mg/L), lactones (40–68 mg/L), acids (42–51 mg/L), ketones (22–28 mg/L), and volatile phenols (6,6–13,5 mg/L). The remaining families: furanic compounds (2,2–4.1 mg/L), norisoprenoids (0.9–1.6 mg/L), IEFAs (0.24–0.79 mg/L), MEFAs (0.10–0.33 mg/L), EEBAs (0.054–0.18 mg/L), HAAs (0.058–0.172 mg/L) and terpenoids (0–0.0146 mg/L) showed the lowest concentration.

3.4. E-nose data

Supplementary Fig. 2 shows the polar plot of 8 sensors response to the set of samples measured related to the three factors. It can be noticed that the sensors give different signal for each sample type, illustrating the discrimination capabilities of the array at a confidence level of 99.99%. The response intensity changes as a consequence of the different headspace composition in sparkling wines. However, no relation among sensors response and the factors studied can be stablished according to this figure.

3.5. Statistical analysis

3.5.1. Univariate analysis

A two-way ANOVA was carried out to the volatilome dataset, and the factors studied were temperature, time and format.

Based on the total contents shown for each chemical family in Table 2, none of the sampling groups is affected simultaneously by all factors. Nevertheless, their interactions may be significant for some families. Alcohols, aldehydes and the group of esters IEFAs are influenced by temperature and ageing time, while this last factor, along with yeast format, also affect EEBAs total content. The temperature contributes to the decrease in HAAs and the increase in lactones, norisoprenoids and furanic compounds. On the other hand, the amount in

Table 2
Average value and (SD[‡]) of the concentration of volatile compounds (mg/L) in sparkling wines for temperature (T), ageing time (t) and yeast format (F) factors and interaction (TxtxF) effect.

N°	Compound	Temperature (T, °C)				Ageing Time (t, months)				Format (F)				*TxtxF*
		10 T	14 T	*p*	VIP	15 M	24 M	*p*	VIP	FC	BIO	*p*	VIP	*p*
Σ Alcohols		404(31)^{b}	363(50)^{a}	*		409(32)^{b}	358(45)^{a}	**		384(63)	382(22)	ns		**
1	Methanol	49(16)	44(11)	ns	0.5	48(8)	45(19)	ns	0.4	47(18)	46(8)	ns	0.03	ns
2	Propan-1-ol	28(2)^{b}	25(3)^{a}	**	1.3	28(2)^{b}	25(3)^{a}	**	1.0	27(4)	27(2)	ns	0.02	***
3	Isobutanol	**57(3)^{b}**	**50(5)^{a}**	**	**1.6**	**56(5)^{b}**	**51(5)^{a}**	*	1.0	**54(7)**	**54(4)**	ns	0.05	***
4	Isoamyl alcohols†	**240(20)^{b}**	**216(29)^{a}**	*	1.2	**247(19)^{b}**	**208(21)^{a}**	***	1.4	**228(36)**	**229(16)**	ns	0.1	*
5	2-Phenylethan-1-ol	**27(3)**	**24(5)**	ns	0.9	**25(5)**	**23(4)**	ns	0.8	**25(6)**	**25(3)**	ns	0.04	**
14	Trans-2-hexen-1-ol	0.2(0.1)	0.2(0.1)	ns	0.9	0.2(0.1)	0.2(0.1)	ns	0.2	0.2(0.1)	0.2(0.1)	ns	0.6	ns
15	Hexan-1-ol	1.6(0.1)	1.6(0.1)	ns	0.6	1.6(0.1)	1.7(0.1)	ns	0.6	1.6(0.1)	1.6(0.2)	ns	0.4	ns
16	2-Ethylhexan-1-ol	0.4(0.2)	0.4(0.1)	ns	0.4	0.5(0.1)^{b}	0.3(0.03)^{a}	***	1.4	0.4(0.2)	0.4(0.1)	ns	0.2	***
17	Decan-1-ol	0.9(0.2)	1.2(0.3)	ns	0.3	1.1(0.2)	1.0(0.4)	ns	0.1	n.f.^{a}	1.1(0.3)^{b}	***	**2.1**	ns
Σ Aldehydes		79(9)^{b}	67(11)^{a}	**		80(11)^{b}	66(9)^{a}	**		74(14)	72	ns		**
6	Acetaldehyde	**79 (9)^{b}**	**66 (11)^{a}**	**	1.4	**79(11)^{b}**	**65(9)^{a}**	**	1.2	**74(14)**	**72(10)**	ns	0.1	**
19	Benzaldehyde	0.04(0.02)	0.04(0.01)	ns	0.2	0.030(0.004)^{a}	0.05(0.01)^{b}	***	**1.6**	0.03(0.01)	0.05(0.02)	ns	0.8	ns
20	Nonanal (μgL^{-1})	**25(25)**	**21(21)**	ns	0.1	**25(25)**	**20(22)**	ns	0.2	**45(8)^{b}**	**15(1)^{a}**	***	**2.1**	**
21	Decanal (μgL^{-1})	**49(41)**	**44(33)**	ns	0.04	**41(43)**	**59(8)**	ns	0.3	**69(17)^{b}**	**0.35(0.01)^{a}**	***	**2.1**	ns
23	4-Hydroxy-2-methoxycinnamaldehyde	0.14(0.05)	0.13 (0.02)	ns	0.1	0.14(0.03)	0.13(0.04)	ns	0.4	0.14(0.03)	0.13(0.04)	ns	0.5	***
24	Benzaldehyde,3,5,bis(1,1-dimethylethyl)-4-hydroxy	0.08(0.03)^{a}	0.10(0.01)^{b}	*	1.1	0.10(0.02)^{b}	0.08(0.01)^{a}	**	1.0	0.08(0.02)^{a}	0.10(0.02)^{b}	*	0.9	ns
Σ Ketones		26(2)	25(2)	ns		26(1)	25(3)	ns		25(3)	26(10)	ns		ns
7	Acetoin	25(2)	25(2)	ns	0.3	26(1)	24(3)	ns	0.7	25(3)	25(2)	ns	0.3	ns
18	2-cyclopenten-1-one,2-hydroxy-	0.6(0.2)^{a}	0.7(0.2)^{b}	**	**1.5**	0.7(0.1)	0.6(0.2)	ns	0.8	0.6(0.1)^{a}	0.7(0.2)^{b}	*	1.1	ns
22	Benzophenone	0.10(0.04)	0.09(0.03)	ns	0.2	0.07(0.02)^{a}	0.11(0.03)^{b}	**	1.3	0.10(0.03)^{b}	0.08(0.03)^{a}	*	0.9	ns
Σ Acids		46(5)	47(5)	ns		44(3)^{a}	49(5)^{b}	**		46(4)	46(6)	ns		ns
25	Hexanoic acid	**2.3(0.2)**	**2.5(0.4)**	ns	0.8	**3(0.3)^{b}**	**2(0.3)^{a}**	**	1.3	**2.4(0.4)**	**2.4(0.3)**	ns	0.3	*
26	Octanoic acid	**40(4)**	**41(5)**	ns	0.2	**38(3)^{a}**	**44(4)^{b}**	**	1.2	**41(4)**	**41(5)**	ns	0.1	ns
27	Decanoic acid	**2.3(0.3)^{a}**	**2.7(0.5)^{b}**	**	**1.5**	**2.3(0.3)^{a}**	**2.8(0.5)^{b}**	**	1.0	**2.4(0.4)**	**2.6(0.6)**	ns	0.6	ns
28	Tetradecanoic acid	0.85(0.02)	0.85(0.01)	ns	0.3	0.9(0.01)^{b}	0.8(0.01)^{a}	*	**1.5**	0.9(0.01)	0.9(0.01)	ns	0.6	**
29	Hexadecanoic acid	n.f.	0.07(0.003)	ns	0.9	n.f.	0.07(0.003)	ns	0.8	n.f.	0.07(0.002)	ns	0.8	***
Σ EEFAs		120(9)	111(16)	ns		121(12)	110(13)	ns		115(17)	115(10)	ns		**
8	Ethyl acetate	**28(2)^{b}**	**24(2)^{a}**	***	**1.7**	**27(2)^{b}**	**24(3)^{a}**	*	1.1	**26(3)**	**26(2)**	ns	0.1	*
9	Ethyl lactate	66(6)	63(12)	ns	0.5	67(8)	61(10)	ns	0.7	64(11)	64(7)	ns	0.1	**
10	Diethyl succinate	23(3)	21(4)	ns	0.7	23(4)	20(3)	ns	0.6	21(4)	22(3)	ns	0.4	ns
30	Ethyl propanoate	0.36(0.04)	0.34(0.03)	ns	0.7	0.34(0.04)	0.35(0.03)	ns	0.5	0.34(0.04)	0.35(0.03)	ns	0.2	***
31	Ethyl butanoate	**0.5(0.1)**	**0.5(0.1)**	ns	0.7	**0.49(0.02)**	**0.5(0.1)**	ns	0.8	**0.5(0.1)**	**0.52(0.04)**	ns	0.6	ns
32	Ethyl hexanoate	**1.4(0.1)**	**1.2(0.1)**	ns	0.2	**1.3(0.1)**	**1.3(0.2)**	ns	0.2	**1.3(0.1)^{b}**	n.f ^{a}	***	**2.1**	***
33	Ethyl octanoate	**1.9(0.3)^{b}**	**1.6(0.2)^{a}**	**	**1.5**	**1.9(0.2)^{b}**	**1.6(0.4)^{a}**	*	0.9	**1.7(0.3)**	**1.7(0.4)**	ns	0.2	***
34	Ethyl-9-decenoate (μgL^{-1})	13(3)^{b}	10(2)^{a}	**	**1.6**	12(3)	11(4)	ns	1.0	12(2)	12(4)	ns	0.3	*
35	Ethyl decanoate (μgL^{-1})	**320(62)^{b}**	**264(58)^{a}**	*	1.2	**281(49)**	**305(82)**	ns	0.5	**302(17)**	**282(91)**	ns	0.6	ns
36	Ethyl dodecanoate (μgL^{-1})	1(1)	1(1)	ns	0.7	1(1)	1(1)	ns	0.2	1.0(0.3)^{a}	2.0(0.2)^{b}	***	**1.9**	***
37	Ethyl tetradecanoate (μgL^{-1})	2(1)	2(1)	ns	0.3	1(1)	2.16(0.04)	ns	0.5	0.2(0.2)^{a}	2.3(0.1)^{b}	***	**2.0**	***
38	Ethyl hexadecanoate	0.01(0.01)	0.01(0.01)	ns	0.1	0.02(0.01)^{b}	0.004 (0.001)^{a}	***	**1.8**	0.01(0.01)	0.01(0.01)	ns	0.4	***
Σ EEBAs		0.10(0.04)	0.12(0.04)	ns		0.08(0.03)^{a}	0.13(0.04)^{b}	*		0.14(0.04)^{b}	0.07(0.02)^{a}	***		ns
39	Ethyl isobutanoate (μgL^{-1})	**26(6)**	**26(3)**	ns	0.05	**22(2)^{a}**	**29(2)^{b}**	***	**1.7**	**26(4)**	**25(5)**	ns	0.2	ns
40	Ethyl −2-Methylbutanoate	**0.02(0.01)**	**0.02(0.01)**	ns	0.7	**0.02(0.01)^{a}**	**0.03(0.02)^{b}**	*	0.9	**0.03(0.01)^{b}**	**0.011(0.003)^{a}**	***	**1.8**	ns
41	Ethyl-3-methylbutanoate	**0.05(0.02)**	**0.06(0.03)**	ns	0.8	**0.04(0.02)^{a}**	**0.06(0.03)^{b}**	*	0.9	**0.07(0.02)^{b}**	**0.03(0.01)^{a}**	***	**1.8**	ns
42	Ethyl-2-methyloctanoate (μgL^{-1})	4.5(0.2)	4.4(0.1)	ns	0.2	4.4(0.1)^{a}	4.5(0.1)^{b}	**	1.3	4.4(0.1)	4.5(0.2)	ns	0.3	*
43	Ethyl-2-phenylacetate (μgL^{-1})	1.0(0.7)^{a}	1.4(0.5)^{b}	*	1.1	1.0(0.4)^{a}	2(0.4)^{b}	***	**1.5**	1(1)	1(1)	ns	0.6	*
Σ MEFAs		0.2(0.1)	0.2(0.1)	ns		0.2(0.1)	0.2(0.1)	ns		0.28(0.04)^{b}	0.12(0.01)^{a}	***		**
44	Methyl acetate	0.1(0.1)	0.2(0.1)	ns	0.5	0.1(0.1)	0.1(0.1)	ns	0.3	0.21(0.04)^{b}	0.05(0.01)^{a}	***	**2.1**	**
45	Pentanoic acid methyl ester (μgL^{-1})	**60(4)^{a}**	**70(4)^{b}**	**	**1.6**	**59(3)^{a}**	**66(4)^{b}**	**	1.4	**62(1)**	**63(1)**	ns	0.1	ns
Σ HAAs		0.15(0.02)^{b}	0.10(0.03)^{a}	***		0.12(0.01)	0.13(0.05)	ns		0.11(0.04)	0.13(0.03)	ns		***
46	Isobutyl acetate (μgL^{-1})	3(1)	4(1)	ns	0.9	4(1)	3(2)	ns	0.8	4(1)^{b}	3.4(0.1)^{a}	***	**1.6**	***
47	Hexyl acetate (μgL^{-1})	20(10)^{b}	15(7)^{a}	*	1.3	23(4)^{b}	11(5)^{a}	***	**1.5**	18(8)	18(6)	ns	0.5	**
48	2-Phenylethyl acetate (μgL^{-1})	68(12)^{b}	49(20)^{a}	*	1.3	70(6)^{b}	40(15)^{a}	***	**1.5**	59(20)	59(19)	ns	0.03	ns
49	Isobornyl acetate	0.06(0.04)	0.04(0.04)	ns	0.6	0.018(0.002)^{a}	0.07(0.04)^{b}	*	**1.5**	0.03(0.03)	0.06(0.04)	ns	0.7	***
Σ IEFAs		0.7(0.1)^{b}	0.5(0.2)^{a}	*		0.7(0.1)^{b}	0.4(0.2)^{a}	***		0.5(0.2)	0.6(0.2)	ns		ns
50	Isoamyl acetate	**0.7(0.1)^{b}**	**0.5(0.2)^{a}**	*	1.3	**0.7(0.1)^{b}**	**0.4(0.1)^{a}**	***	**1.5**	**0.5(0.2)**	**0.6(0.2)**	ns	0.2	ns
Total Esters		121(9)	112(16)	ns		122(12)^{b}	111(13)^{a}	*		116(17)	116(10)	ns		**
Σ Lactones		47(9)^{a}	64(6)^{b}	***		60(11)	51(10)	ns		54(12)	57(11)	ns		*
51	γ-Crotonolactone	**45(10)^{a}**	**61(7)^{b}**	**	**1.9**	**57(12)**	**50(10)**	ns	0.9	**49(11)**	**57(11)**	ns	0.75	*
52	γ-Butyrolactone	**3.8(0.4)^{a}**	**6(1)^{b}**	**	0.5	**5(2)**	**5(1)**	ns	0.3	**5(1)^{b}**	n.f. ^{a}	***	**2.1**	***
53	Caprolactone (μgL^{-1})	12(15)	6(3)	ns	0.4	14(13)^{b}	3(2)^{a}	***	**1.5**	12(15)	6(2)	ns	0.1	ns
54	γ-Nonalactone (μgL^{-1})	7.0(0.3)	12(18)	ns	0.6	7.1(0.3)	13(19)	ns	0.4	7.0(0.3)	12(18)	ns	0.5	ns
55	γ-Decalactone (μgL^{-1})	5(1)	5(1)	ns	0.7	4.3(0.3)^{a}	12(3)^{b}	***	**1.9**	5(1)	5(1)	ns	0.2	ns
Σ Terpenoids		0.01(0.00)	0.01(0.00)	ns		0.01(0.00)	0.01(0.00)	ns		n.f. ^{a}	0.01(0.00)^{b}	***		ns
56	Nerolidol, trans (μgL^{-1})	13(1)	13(1)	ns	0.03	14.4(0.3)	11.9(0.2)	ns	0.2	n.f. ^{a}	13(1)^{b}	***	**2.1**	ns
Σ Norisoprenoids		0.9(0.1)a	1.2(0.2)^{b}	***		1.0(0.1)	1.2(0.3)	ns		1.0(0.2)	1.1(0.3)	ns		ns
57	Vitispirane	0.4(0.1) a	0.7(0.2) ^{b}	***	**1.9**	0.5(0.1)	0.7(0.3)	ns	0.6	0.5(0.2)	0.6(0.3)	ns	0.8	*
58	β-Damascenone	0.5(0.1)	0.5(0.1)	ns	0.8	0.5(0.1)	0.5(0.1)	ns	0.2	0.6(0.1)^{b}	0.5(0.1)^{a}	*	1.2	ns

(*continued on next page*)

Table 2 (*continued*)

N°	Compound	Temperature (T, °C)				Ageing Time (t, months)				Format (F)				*TxtxF*
		10 T	14 T	*p*	VIP	15 M	24 M	*p*	VIP	FC	BIO	*p*	VIP	*p*
	Σ Volatile phenols	9(2)	11(3)	ns		10(3)	10(3)	ns		10(3)	10(2)	ns		***
59	*Guaiacol*	**0.10(0.04)^{a}**	**0.15(0.03)^{b}**	**	**1.6**	**0.13(0.04)**	**0.12(0.05)**	ns	0.3	**0.10(0.03)^{a}**	**0.14(0.04)^{b}**	*	0.9	***
60	4-Ethyl-2-methoxyphenol	**0.3(0.1)^{a}**	**0.4(0.1)^{b}**	*	1.4	**0.4(0.1)**	**0.4(0.1)**	ns	0.6	**0.3(0.1)**	**0.4(0.1)**	ns	0.1	**
61	Syringol	0.04(0.02)	0.03(0.01)	ns	0.5	0.04(0.02)^{b}	0.021(0.003)^{a}	***	**1.7**	0.04(0.02)^{b}	0.03(0.01)^{a}	*	0.9	ns
62	Phenol, 2 methoxy-4-(1 propenyl)-	0.6(0.4)	0.5(0.3)	ns	0.5	0.7(0.5)	0.5(0.2)	ns	0.2	0.3(0.1)^{a}	0.8(0.3)^{b}	***	**1.9**	ns
63	Phenol, 2,4-bis(1,1-dimethylethyl)	8(2)	10(3)	ns	0.9	9(2)	9(3)	ns	0.2	9(3)	9(2)	ns	0.3	***
	Σ Furanic compounds	3(1)^{a}	4.0(0.4)^{b}	***		4(1)	3(1)	ns		3(1)	4(1)	ns		*
64	(Furan-2-yl) methanol	1.2(0.4)^{a}	1.9(0.3)^{b}	**	**1.8**	1.7(0.5)	1.4(0.5)	ns	0.6	1.3(0.5)^{a}	1.8(0.4)^{b}	*	1.0	**
65	Furan-2-carbaldehyde	**1.3(0.2)**	**1.5(0.3)**	ns	0.9	**1.5(0.3)**	**1.3(0.3)**	ns	0.5	**1.5(0.3)**	**1.4(0.2)**	ns	0.1	**
66	5-(Hidroxymethyl)-2-furaldehyde	0.3(0.1)^{a}	0.5(0.1)^{b}	**	1.4	0.5(0.1)^{b}	0.3(0.1)^{a}	*	1.16	0.4(0.1)	0.5(0.1)	ns	0.8	***
67	Ethyl furoate	0.02(0.01)^{a}	0.03(0.01)^{b}	**	**1.6**	0.018(0.004)^{a}	0.03(0.01)^{b}	**	1.4	0.02(0.01)	0.02(0.01)	ns	0.4	ns
	Σ Polyols	329(66)	284(50)	ns		329(76)	285(33)	ns		302(46)	312(76)	ns		*
11	2.3-Butanediol (levo)	268(53)	232(42)	ns	1.0	268(62)	231(28)	ns	0.7	246(38)	254(62)	ns	0.1	*
12	2.3-Butanediol (*meso*)	61(13)	53(9)	ns	1.0	61(14)	52(6)	ns	0.7	56(8)	58(15)	ns	0.1	**

10 T: sparkling wines elaborated at 10 °C. 14 T: sparkling wines elaborated at 14 °C. 15 M: sparkling wines 15 months aged. 24 M: sparkling wines 24 months aged. FC: sparkling wines elaborated with free yeast cells. BIO: sparkling wines elaborated with bio-immobilized yeast cells. Significant level: ns = non-significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. n.f. = not found. EEFAs: Ethyl esters of fatty acids: EEBAs: Ethyl esters of branched acids, MEFAs: Methyl esters of fatty acids; HAAs: Higher alcohols acetates: IEFAs: Isoamyl esters of fatty acids. VIP: variable importance in projection. In bold letters, the concentrations of compounds having the Odour Activity Value (OAV greater than 1) and the potential markers with VIP ≥ 1.5. † Isoamyl alcohols = 2-methylbutanol + 3-methylbutanol. ‡ standard deviation calculated by grouping the samples for which the analysed factor has the same value.

carboxylic acids increase with ageing time while MEFAs and terpenoids contents are yeast-format dependent. Although no significant differences have been found in the remaining chemical families related to the three factors, some specific compounds are affected by them.

The decrease observed in the alcohols family with respect to temperature and ageing is caused by propan-1-ol (2), isobutanol (3) and isoamyl alcohols (4), meanwhile 2-ethylhexan-1-ol (16) content was affected only by ageing time (Table 2). However, no changes for these compounds were described in literature for sparkling wines obtained with similar ageing time (Riu-Aumatell et al., 2006; Torrens et al., 2010). In contrast, only the content in decan-1-ol increases in wines from yeast biocapsules (BIO).

Concerning to aldehydes, the main variation observed between 10 T and 14 T wines were due to acetaldehyde (6) content, which decreased the 16.5% at 14 °C temperature. This compound also decreases in 24 M aged wines, in accordance with the results of Torrens et al. (2010). With regards to yeast format, BIO wines displayed lower content in nonanal (20) and decanal (21), in agreement with the results obtained by Lopez de Lerma et al. (2018). On the other hand, changes in ketones 18 and 22 contents were observed in relation to some of the three studied factors, while acetoin (7) did not depend of any.

Carboxylic acids content increased by more than 11% in 24 months aged wines, being octanoic acid the main contributor for this trend. The remaining factors do not affect this chemical group, except to decanoic acid whose content increases at 14 °C. Previous studies carried out by authors during in bottle second fermentation showed similar tendency, as a response of yeasts to the CO_2 over-pressure stress (Martinez-Garcia et al., 2017; Martinez-Garcia et al., 2020).

The total content in ester family is only affected by ageing time, showing a decrease of 9% from 15 to 24 months (Table 2). The greater contributor to this family is EEFAs group, whose content is not significantly affected by the three factors analysed. Nevertheless, the values in ethyl acetate and ethyl octanoate decreases as a consequence of both temperature and ageing time. Similar trend is observed in ethyl-9-decenoate content with temperature (-30% of decrease), ethyl hexadecanoate with ageing (-80%), and ethyl hexanoate, which is not detected in biocapsules wines. This can be explained either by the faster hydrolysis that longer-carbon chain compounds suffer or by faster retention that high hydrophobic molecules have on lees surface (Gallardo-Chacon, Vichi, Lopez-Tamames, & Buxaderas, 2010). On the other hand, BIO sparkling wines displayed the greater content in ethyl dodecanotate and ethyl tetradecanoate.

Ethyl esters of branched acids (EEBAs) displayed an increment with ageing time (from 80 μg/L to 130 μg/L), in accordance to the results from (Perez-Magarino et al., 2015). This group is also affected by yeast format, being registered higher levels in wines (FC24M) obtained with free cells and 24 months aged (Supplementary Table 2). Furthermore, all the compounds identified are ageing dependent, while 2- and 3-methylbutanoic acids and phenylacetic acid ethyl esters (40, 41 and 43) are also dependent of yeast format and/or temperature. Ruiz-Moreno et al. (2017) described that ethyl isobutyrate, ethyl-2-methylbutyrate and ethyl isovalerate increase in a 1.9-fold in sparkling wines aged from 0 to 9 months, not being observed changes for ethylphenyl acetate with ageing, in contrast to the results here exposed.

Yeast format also contributes to the decrease of the MEFAs total content, being BIO sparkling wines those with lower levels. Methyl acetate stands out, although pentanoic acid methyl ester was also dependent of both temperature and ageing. With regard to these last two factors, highlight IEFAs with isoamyl acetate as main responsible, whose observed decrease has been also described by Riu-Aumatell et al. (2006) and Torrens et al. (2010).

Higher alcohols acetates (HAAs) content decreases mainly by temperature, in contrast with the results exposed by Giovenzana et al. (2016). From the four acetates quantified (46,47,48,49), only hexyl acetate and 2-phenylethyl acetate changed significantly ($p \leq 0.05$) their contents in relation to this factor. These compounds along with isobornyl acetate are also dependent on ageing, in accordance with other authors results, although this latter shows an opposite trend as it increases with ageing (Riu-Aumatell et al., 2006; Torrens et al., 2010; Ruiz-Moreno et al., 2017). Finally, only isobutyl acetate showed lower content in BIO wines

The increase observed in lactones content with temperature is mainly caused by γ-crotonolactone (51) and γ-butyrolactone (52). Additionally, previous studies carried out by authors show that this family is influenced by CO_2 overpressure, displaying a decrease for some yeast strain (Martinez-Garcia et al., 2020). The content in γ-butyrolactone is also affected by yeast format, not being found in BIO sparkling wines. Concerning ageing factor, caprolactone and γ-decalactone showed the opposite trend.

Varietal volatile compounds, such as norisoprenoids and terpenoids exhibited an increase with temperature and yeast format respectively (Table 2). Among them, only vitispirane has been proposed as potential marker for aged sparkling wines (Riu-Aumatell et al., 2006). However, no changes were observed regarding this factor in our study.

Finally, some volatile phenols are either affected by temperature (60), format (62) or both (59), being only syringol (61) affected by ageing and format. Furthermore, wines elaborated at 14 °C showed higher contents in furanic compounds 64, 66 and 67 mainly. Ethyl furoate (67) and (furan-2-yl) methanol (64), also increase their contents with the ageing time and yeast format, respectively. In contrast, compound 5-(Hidroxymethyl)-2-furaldehyde (66) decreased in 40% with ageing, in line with the results observed in Table 1 for A_{280} values. This compound was proposed by Serra-Cayuela et al. (2014), as a "better time and temperature marker, than A_{420} or phenolics, as a consequence of its linearity with time and sensitivity to temperature changes".

3.5.2. Multivariate analysis

3.5.2.1. Volatilome Heat-map. The first approach was to build a heat map through volatilome dataset to provide a comprehensive and easy to understand overview about the effects of temperature, ageing and format on each quantified compound (Supplementary Fig. 3). The square Euclidean distance and Ward's method were used as grouping rule and as a measure of the proximity among samples. According to this figure, compounds (in columns) were grouped in four clusters and their relative contents coloured in blue and red, indicating low and high levels, respectively. Regarding to the compounds of cluster 1, 24 M wines displayed the lowest content compared to 15 M wines obtained at 10 °C (FC15M10T and BIO15M10T). Also, the compounds belonging to cluster 2, showed low contents in general, exception made for the wines obtained at low temperatures and long ageing times. Furthermore, the amounts of compounds 23, 27, 29, 45, 57 and 67, were higher for BIO24M14T wines, which are characterized by fruity, floral, balsamic and also fatty aroma (Supp. Table 1). With regard to cluster 3, wines made by BIO format had the largest levels for these compounds, compared to FC format that showed low content in compounds: 17, 36, 37, 56 and 62 that are related to chemical, fatty and empyreumatic aromas (Leffingwell, 2020; Welke, Zanus, Lazzarotto, & Zini, 2014). The opposite trend was observed for the compounds of cluster 4, where BIO wines displayed lower values in this group, being the maximum record for FC24M14T wines.

3.5.2.2. Principal component analysis (PCA) for volatilome and E-nose data. Principal component analysis (PCA) is widely used for diminishing the dataset dimension, allowing to visualize easily the differences among sample types. Regarding this, to study the effect of temperature (T), ageing (t) and format (F) on the volatilome (66 variables) and also the discriminant capacity of the eight E-nose sensors, a PCA from each dataset was built. After the feature extraction, a pre-processing was performed to the data (mean center and scaling).

Fig.1 shows the PCA obtained for both data matrices. Regarding to volatilome model, seven components were found to be relevant and explained more than 88.6% of total variance, meanwhile the first two component explain about 50% of the total variance. The samples scores and the loadings plot for PC1 and PC2 are shown in Fig. 1A.1 and A.2, respectively. It can be noticed this dataset is clearly separated, allowing an easy differentiation among the samples in relation to temperature, time and format (Fig. 1A.1). Samples on the left have negative scores for PC1 and corresponds to wines 24 months aged (24 M), while wines with 15 months aged (15 M) are mainly grouped on the right of PC1.

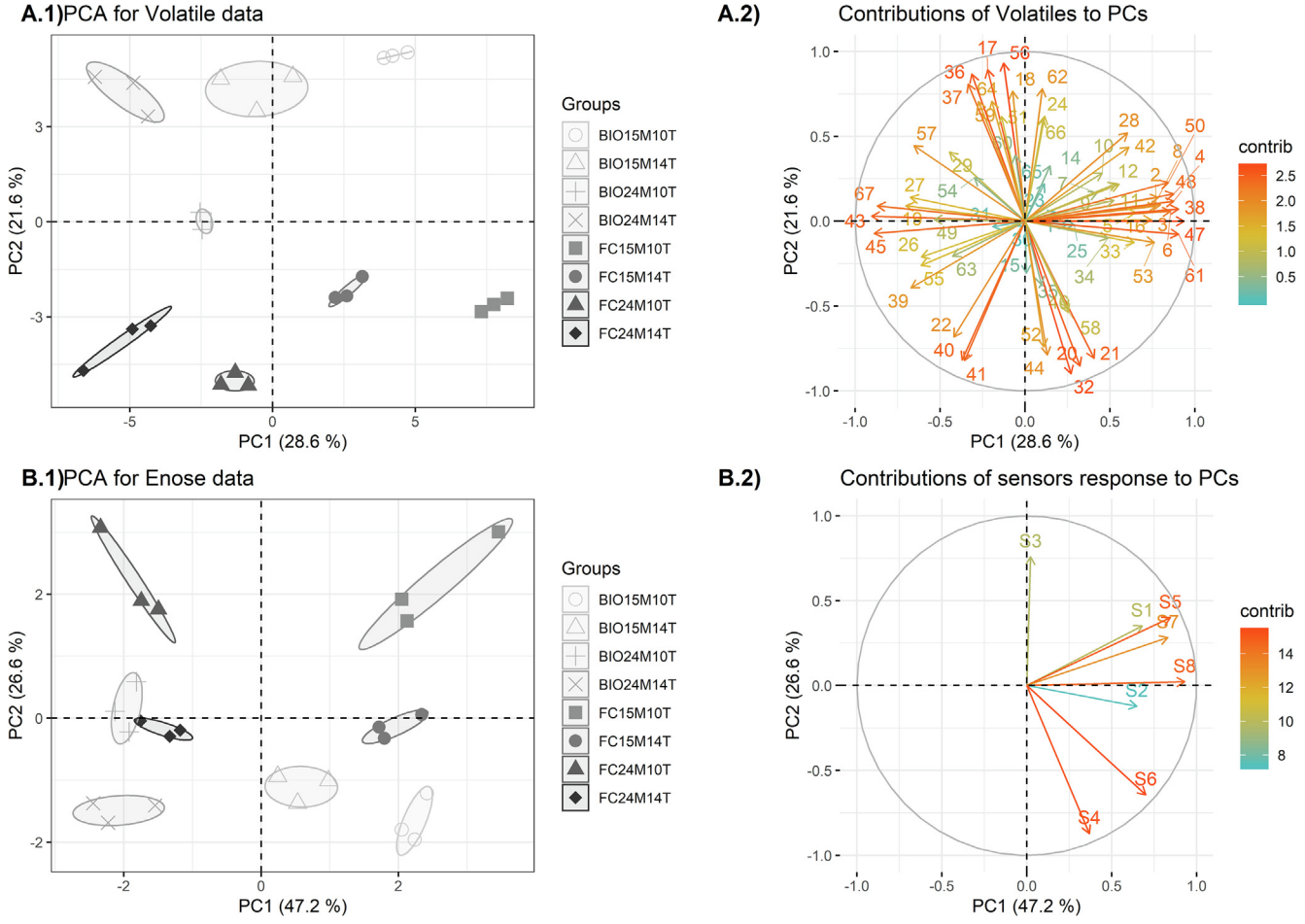

Fig. 1. Principal component analysis performed with volatile aroma compounds (A) and E-nose datasets (B). Figs. A.1 and B.1 shows the scores plot and Figs. A.2 and B.2 shows the loadings plots for volatilome and E-nose data, respectively. For identification of wine samples and compounds see Table 2.

Table 3
Statistics results of the PLS-DA models from E-nose data according to the temperature (T), ageing time (t) and format (F) factors.

Classes[a]	Temperature (T)[b]		Ageing Time (t)[c]		Format (F)[d]	
	10 T	14 T	15 M	24 M	FC	BIO
Calibration step						
Sensitivity (cal)	100%	100%	100%	100%	100%	100%
Specificity (cal)	100%	100%	100%	100%	100%	100%
RMSEC	0.143	0.143	0.157	0.157	0.173	0.173
R^2	0.918	0.918	0.902	0.902	0.880	0.880
Cross-validation step						
Sensitivity (CV)	83.3%	100%	100%	100%	100%	100%
Specificity (CV)	100%	83.3%	100%	100%	100%	100%
RMSECV	0.298	0.298	0.167	0.167	0.238	0.238
R^2	0.698	0.698	0.904	0.904	0.835	0.835

[a] Sensitivity: recall or true positive rate. Specficity: true negative rate. RMSEC: Root mean squares error at the calibration step. RMSECV: Root mean squares error at the cross-validation step. R^2: Regression coefficient.

[b] 10 T: sparkling wines elaborated at 10 °C. 14 T: sparkling wines elaborated at 14 °C.

[c] 15 M: sparkling wines 15 months aged. 24 M: sparkling wines 24 months aged.

[d] FC: sparkling wines elaborated with free yeast cells. BIO: sparkling wines elaborated with bio-immobilized yeast cells.

Temperature effect is clearly visualized by comparing wines with the same aging time because those obtained at 10 °C showed higher scores for PC1. In addition, the effect of yeast format can be observed by PC2 values, where samples obtained with biocapsules (BIO) are located on the top of PC2 and those obtained with free cells (FC) are located on the bottom. To our knowledge, this is the first time in which a PCA from the volatilome allows to differentiate sparkling wines according to the factors temperature, ageing time and yeast format. Other authors use this tool to visualize the effect of individual factors such as: ageing time (Riu-Aumatell et al., 2006) and yeast format (Puig-Pujol et al., 2013; Lopez de Lerma et al., 2018), not being found studies related to temperature.

Fig. 1A.2 shows that PC1 correlates positively, mainly with 4 (isoamyl alcohols), 6 (acetaldehyde), 48 (2-phenylethyl acetate), 50 (isoamyl acetate), 61 (syringol) and negatively with volatiles numbered 43 (ethyl-2-phenylacetate), 45 (pentanoic acid methyl ester) and 67 (ethyl furoate). All of them were mentioned before as dependent on the ageing factor. In contrast, PC2 correlates positively with the compounds: 17 (decan-1-ol), 36 (ethyl tetradecanoate), 37 (ethyl hexadecanoate) and 56 (nerolidol), and negatively with 20 (nonanal), 21 (decanal), 32 (ethyl hexanoate), 40 (ethyl-2-methylbutanoate) and 41 (ethyl-3-methylbutanoate), whose changes in concentration are mainly dependent on the format.

With regard to PCA of E-nose data, three components were found to be relevant and explained more than 86% of total variance. Principal component 1 (PC1) explained 47.2% of the overall variance and the sensors (S) numbered 5 (Co-*p*-TPP) ,7 (Co-TPP) and 8 (Ru-TPP) had the strongest effect on it (Fig. 1B.2). Some studies have investigated the role of metal ion in metalloporphyrin and an association between sensors and volatiles can be observed, through the use of an adsorption isotherm model and the proper data treatment (Catini et al., 2016; Saevels, Berna, Lammertyn, Di Natale, & Nicolai, 2004). Despite this, in the current study the correlation between sensors response and the content of the different chemical families was considered as a tentative approach. Thus, related to the sensors mentioned above for PC1, the obtained correlation coefficients (r) ranged 0.7–0.86 ($p < 0.001$) for the families of aldehydes, HAAs and IEFAs. Saevels et al. (2004) found that S5 and S8 had more affinity to alcohols and esters, respectively. In addition, PC2 accounted 26.6% of the variance, being sensors 3, 4 and 6 the greatest contributors. From them, S3 (Sn-TPP) had the lowest r values (0.44–0.45) at $p < 0.05$ and correlated positively with EEBAs and MEFAs esters group. Sensor 4 (Rh-TPP) correlated positively ($p < 0.001$) with lactones (r: 0.72), volatile phenols (r: 0.68) and furanic compounds (r: 0.82), meanwhile S6 (Cr-TPP) was negatively correlated with EEBAs (r: −0.71). This latter, along with sensor 2, is also characterised by high affinity for butyl 2-methylbutanoate compared to the other volatiles, according to Saevel et al., (2004).

Based on the score plots for this dataset (Fig. 1B.1) the distribution of the samples is similar to those obtained with the volatilome, although there are slight differences. Samples scores for PC1 grouped the wines in relation to the time, locating wines aged for 15 months to the right and those with 24 months ageing, to the left of this axis. Differences due to format and temperature can be appreciated along PC2 axis, showing the wines obtained with free cells (FC) positive or near to zero scores and negative values those obtained with biocapsules (BIO). Temperature effect is visualized by comparing PC2 scores for wines elaborated with the same yeast format and ageing time, showing wines at 10 °C the higher values. Nevertheless, this model has a worse discrimination between 24 months aged wines obtained with each yeast contact mode at temperatures of 10 and 14 °C (FC24M14T and BIO24M10T). Other authors found that E-nose based in quartz microbalances was able to differentiate wines obtained under similar conditions in a Protected Denomination of Origin (Lopez de Lerma et al., 2013), or to follow the changes in aroma profiles during grape dehydration process at different temperatures (Santonico et al., 2010). Related to ageing time classification, Lozano et al. (2008) also used of resistive metal oxide semiconductor (MOS) sensors in still wines aged in different types of oak barrels at 0, 3, 6 and 12 months. According to our results, the models obtained by using volatilome data appear to have a higher separation power among the wines than those obtained with E-nose dataset. The low cumulative variance shown in this model for the first two components, may be explained by the inclusion of those variables that not having discrimination power among the samples, contribute to the increase in background noise.

3.5.2.3. PLS-DA models of E-nose data and potential volatile markers. Three PLS-DA models were built (Table 3) through the E-nose data arranged in a 24 × 8 matrix. Each row corresponded to the samples and each column to the sensors signal. The first model was built based on temperature effect, labelling sparkling wines obtained at 10 °C (10 T) as class 0 and 14 T sparkling wines as class 1. The second PLS-DA model was built based on ageing effect, in which 15 months aged wines (15 M) were labelled as class 0 and 24 months wines (24 M) as class 1. Finally, in relation to the yeast format, the class 0 was assigned to the wines obtained with free cells (FC) and class 1 those obtained with biocapsules (BIO). Several pre-processing techniques were evaluated, being 'autoscaling and centering' the one that achieved the highest prediction ability because they remove any influence due to different ranges of sensors responses and also diminish the influence of a non-homogeneous sensor deposition (Lozano et al., 2008; Catini et al., 2016). For all models the re-sampling methodology used was leave one out cross validation (LOOCV) and the number of latent variables (LVs) were selected according to the lowest prediction error (RMSECV) criteria. Thus, for temperature, ageing and format discriminant models, were considered as optimal 7, 2 and 3 LVs, respectively. According to the results in Table 3, the sensitivity (true positives) and specificity (true negatives) rates were 100% for ageing time and format models. Lower values were obtained for temperature models, that were characterised with higher error (0.298) and lower regression coefficient (0.698) for the internal validation. There are not studies based on the application of E-nose for sparkling wine discrimination and those applied on still wines are focused on their differentiation according to their ageing time, being the classification rate of 87 to 97% by using nonlinear techniques (Lozano et al., 2008).

The PLS-DA was also applied to the volatilome dataset in order to detect potential marker compounds. Regarding this the same

methodology described above was applied on a 24 rows (samples) × 66 columns (volatile compounds) matrix. The PLS-DA models (results no shown) were built with 6, 5 and 2 LVs to study the temperature, ageing and format effect, respectively. For this dataset, the sensitivity and specificity rates were 100%, showing RMSECV values of 0.078, 0.110 and 0.055 and regression coefficients 0.978, 0.958 and 0.989 (for ageing time, temperature and yeast format, respectively). This evidenced better results for this dataset compared to models exposed in Table 3 for E-nose dataset.

The assignation of potential markers to each factor studied, was established following the methodology introduced by Munoz-Redondo et al. (2017) and Ruiz-Moreno et al. (2017). The criterion is based on selecting compounds with a variable importance projection (VIP) values (calculated from the PLS-DA) equal or higher than 1.5 and a category α of the factorial analysis of variance with a Fisheŕs least significant level of 0.01 (Table 2). With regard to temperature markers compounds, isobutanol (3), the EEFAs ethyl acetate (8), ethyl octanoate (33) and ethyl-9-decenoate (34), can be considered as candidate markers of 10 T class. Likewise, 2- cyclopentene-1-one, 2 hydroxy (18), γ-crotonolactone (51), vitispirane (57), guaiacol (59), (furan-2-yl) methanol (64) were selected as candidate for the 14 T class. For this class, some compounds with VIP values greater than 1.5 such as decanoic acid (27), and pentadecanoic acid methyl ester (45), were rejected because they shared α category with the other factors studied. However, 5-(hidroxymethyl)-2-furaldehyde (66) displayed VIP coefficients close to 1.5 and non-shared category α in the univariate analysis and may be also considered as potential marker linked to each named factor, in accordance to Serra-Cayuela et al. (2014).

Among the ageing time markers, it is important to highlight that most of them belonged to the ester family. Thus, ethyl hexadecanoate (38), hexyl acetate (47), 2-phenylethyl acetate (48), isoamyl acetate (50), caprolactone (53) and syringol (61) were obtained as candidate markers of the 15 M class. In contrast, benzaldehyde (19), ethyl isobutanoate (39), ethyl-2-phenylacetate (43) and decalactone (55), were the potential volatile markers of 24 M class. These results are in agreement with Riu-Aumatell et al. (2006) and Ruiz-Moreno et al., (2018). Additionally, the isoamyl alcohols (22), 2-ethenylhexan-1-ol (16) and hexanoic acid (25) for 15 M class, and benzophenone (22), ethyl-2-methyloctanoate (42) for 24 M class, displaying a VIP value close to 1.5, may also be considered as potential markers of ageing time factor.

Regarding to the yeast format, and according the selection criteria described above (VIP values ≥ 1.5 and α ≤ 0.01), two aldehydes (20 and 21), ethyl hexanoate (32), two EEBAs (40 and 41), methyl acetate (44), isobutyl acetate (46) and γ- butyrolactone (52) were selected as markers for wines elaborated with free cells (FC) and decan-1-ol, two EEFAs (36 and 37), nerolidol-trans (56) and phenol,2-methoxy-4-(1 propenyl)- (62), were considered as markers for BIO wines.

Among the volatile markers above mentioned, only a few may be considered as contributors to the sensory differences of wines. Thereby, isobutanol, ethyl acetate, ethyl octanoate, γ-crotonolactone and guaiacol for temperature and ethyl isobutanoate for ageing, displayed concentrations above the odour perception threshold (OPT). Likewise, compounds 20, 21, 32, 40, 41, and 52 showed higher levels than their OPT in FC wines. Meanwhile, none of the markers for BIO sparkling wines exceeded their thresholds values.

3.5.2.4. Multiple variable analysis of odorant series. A fingerprint through the odorant series (OS) values for each sparkling wine was built by applying a Multiple Variable Analysis (MVA) to the previously scaled and normalized dataset. This methodology provides an objective and useful way to establish the cause-effects relationships (Martinez-Garcia et al., 2017; Martinez-Garcia et al., 2020). To give a more accurate result, those compounds without their specific calibration curve or perception threshold, were excluded from this analysis (17, 18, 22, 23, 24, 34, 42, 49, 58, 62 and 63). One-way ANOVA was also made to identify significant differences among each odorant series from the eight group of samples.

Fig. 2 shows an eight-vertex polygonal shape obtained from the OAV for each volatile compound, grouped into eight odorant series (OS): 1. Chemical; 2. Fruity; 3. Floral; 4. Fatty; 5. Balsamic; 6. Vegetal; 7. Empyreumatic; 8. Spicy. The shape of fingerprints obtained for the 15 months aged wines at 10 °C, in both yeast formats (FC15M10T and BIO15M10T) are very similar or slightly higher to their counterparts obtained at 14 °C (FC15M14T and BIO15M14T). However, this trend is not observed in 24 months aged wines, where the shapes are very different from each other. Furthermore, FC15M sparkling wines showed the largest fingerprint, meanwhile BIO24M10T showed the smallest

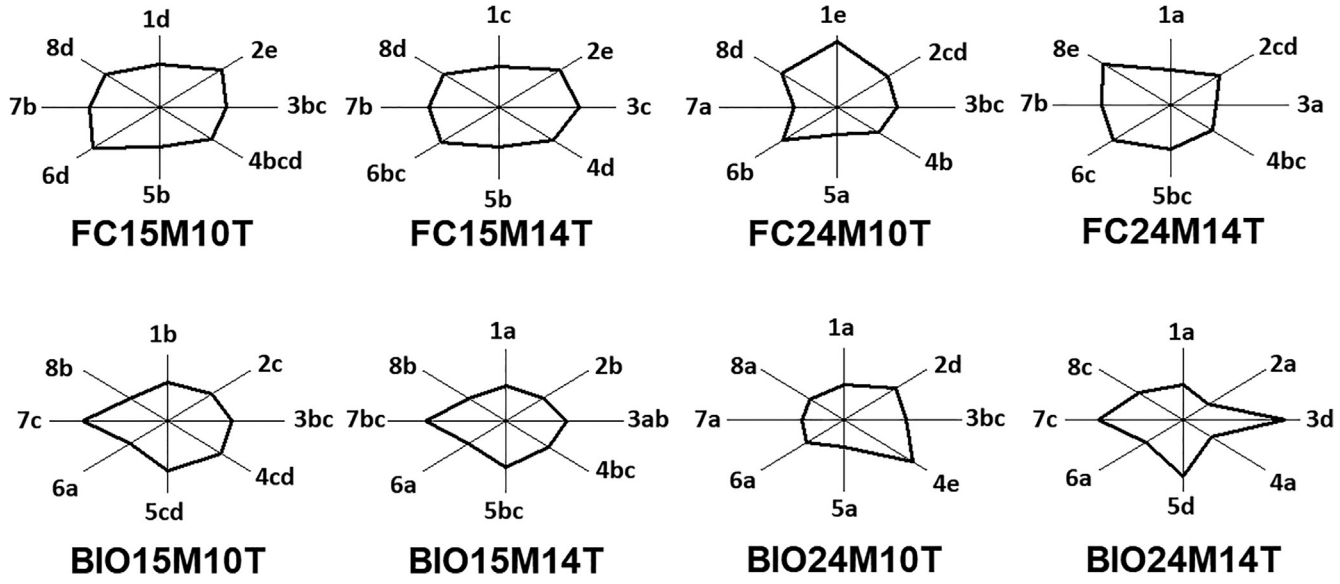

Fig. 2. Footprints obtained by multivariate data analysis of free aroma compounds grouped in odorant series. Each ray in the polygon corresponds to one odorant series. The distance from the centre to each vertex corresponds to the value of each group. The end of the ray is the mean value plus three standard deviations and the centre the mean minus three standard deviations. Aroma series are: 1. Chemical; 2. Fruity; 3. Floral; 4. Fatty; 5. Balsamic; 6. Vegetal; 7. Empyreumatic; 8. Spicy VIII. [a. b. c. e] Different letters in the series indicate statistical differences of the scaled data at 0.05 level according to Fisher's least significant difference. For identification of wine samples see Table 2.

Table 4
Mean of odorant activity values (standard deviations[‡]) of aroma compounds grouped in eight odorant series (OS) analysed in sparkling wines for temperature (T), ageing time (t) and format (F) factors and interaction (TxtxF) effect.

		Temperature (T, °C)			Ageing Time (t, months)			Format (F)			*TxtxF Interaction*
N°	OS	10 T	14 T	*p*	15 M	24 M	*p*	FC	BIO	*p*	*p*
1	*Chemical*	33(14) b	23(3) a	*	26(4)	30(16)	ns	34(14) b	22(2) a	***	***
2	*Fruity*	508(53)	448(94)	ns	503(76)	454(80)	ns	532(45) b	425(73) a	***	**
3	*Floral*	3.9(0.2)	4(1)	ns	4.0(0.4)	4(1)	ns	3.8(0.4)	4(1)	ns	***
4	*Fatty*	481(54) b	432(42) a	*	468(27)	445(71)	ns	455(30)	459(71)	ns	*
5	*Balsamic*	114(38) a	152(28) b	**	142(22)	123(49)	ns	119(27)	146(44)	ns	ns
6	*Vegetal*	8(8)	7(6)	ns	9(8)	7(6)	ns	14(3) b	1.4(0.1) a	***	**
7	*Empyreumatic*	190(53) a	234(27) b	*	234(24) b	190(54) a	*	199(36)	225(54)	ns	ns
8	*Spicy*	28(9)	32(10)	ns	28(8)	32(11)	ns	38(5) b	21(3) a	***	ns

a,b. Different letters in the same row indicate statistical differences of the normalized and scaled data at 0.05 level according to Fisher's least significant difference method. Significant level: ns = non-significant, * = $p < 0.05$, ** = $p < 0.01$, ***= $p < 0.001$. 10 T: sparkling wines elaborated at 10 °C. 14 T: sparkling wines elaborated at 14 °C. 15 M: sparkling wines 15 months aged. 24 M: sparkling wines 24 months aged. FC: sparkling wines elaborated with free yeast cells. BIO: sparkling wines elaborated with bio-immobilized yeast cells. [‡] standard deviation calculated by grouping the samples for which the analysed factor has the same value.

fingerprints. As general rule, FC sparkling wines registered higher values in Chemical (1), Fruity (2), Vegetal (6) and Spicy (8) series and low levels in Balsamic (5) and Empyreumatic (7) series, compared to BIO sparkling wines.

To study the effect of each factor (without considering their interaction), two-way ANOVA was applied to the odorant series dataset (Table 4). Thus, with regard to temperature, 10 T wines exhibited significant higher values in 1 and 4 series at $p \leq 0.05$ level and lower values in 5 and 7 series ($p \leq 0.01$ and $p \leq 0.05$), non being observed significant changes for the remaining series. Regarding ageing time, only significant changes ($p < 0.01$) for Empyreumatic series pointed out, being lower in 24 M wines. Concerning the yeast format, BIO wines revealed lower values ($p \leq 0.001$) for Chemical, Fruity, Vegetal and Spicy series. This diminution may be interpreted as an effect of adsorption of yeast biocapsules during the ageing time, which should be studied. Finally, the interaction effect between factors was significant ($p < 0.001$) for Chemical, Fruity, Floral, Fatty, Vegetal and Spicy series.

4. Conclusions

The univariate and multivariate statistical analysis applied to volatilome dataset show that HAAs, lactones, norisoprenoids and furanic compounds are dependent on temperature, carboxylic acids on aging time, whereas MEFAs and terpenoids on yeast format. Alcohols, aldehydes and IEFAs are influenced by temperature and aging, while this last factor, along with yeast format, also affect EBBAs total content. The variable importance projection values (VIP) obtained through the PLS-DA models and significance level $\alpha \leq 0.01$ allow to establish that twelve volatiles are significantly dependent on yeast format, ten of temperature and eleven of "on lees" ageing time. From them, only isobutanol, ethyl acetate, γ-crotonolactone and guaiacol for temperature and ethyl isobutanoate for aging, stood out as potential volatile markers with OAV > 1. The twelve compounds selected as markers of yeast factor do not exceed their perception threshold.

Regarding the responses of an electronic nose, PLS-DA allows to classify the 100% of the sparkling wines concerned the yeast format and ageing time and the 83% of wines according to the temperature used in their elaboration process. Likewise, the classification rates obtained by applying the same statistic procedure to the volatilome dataset were 100% for all factors.

Finally, tentative correlations among the E-nose sensors and the chemical families of volatiles are established, revealing as the E-nose is a suitable tool for applications in the oenology field. Further studies are needed to make more comprehensive the interactions volatiles-sensors and the interaction effects of temperature-time-yeast format in the composition and quality of sparkling wines.

CRediT authorship contribution statement

Rafael Martínez-García: Formal analysis, Writing - original draft. **Juan Moreno:** Conceptualization, Writing - review & editing, Resources. **Andrea Bellincontro:** Conceptualization. **Luna Centioni:** E-Nose training, analysis. **Anna Puig-Pujol:** Conceptualization, Funding acquisition. **Rafael A. Peinado:** Conceptualization, Writing - review & editing, Methodology. **Juan Carlos Mauricio:** Conceptualization, Writing - review & editing. **Teresa García-Martínez:** Writing - review & editing.

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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Appendix A. Supplementary data

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