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Towards a better understanding of the evolution of odour-active compounds and the aroma perception of sparkling wines during ageing

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ABSTRACT

A native veil-forming yeast and a commercial yeast strain were used to elaborate sparkling wines by the Champenoise method with a grape variety traditionally used for the production of still wines. Wines aged on lees for fifteen months were sampled at five points and their physicochemical and sensory indices were analysed. Unsupervised and supervised statistical techniques were used to establish a comparison between 81 volatile compounds and eight odour descriptors (chemical, fruity, floral, fatty, balsamic, vegetal, empyreumatic and spicy). Principal component analysis of both datasets showed good separation among the samples in relation to ageing time and yeast strain. By using a partial least squares regression-based criterion, 38 odour active compounds were selected as the most influential for the ageing factor and out of them, only 27 were unique to certain aroma descriptors. These results contribute to a better understanding of the aroma perception of sparkling wines.

1. Introduction

The international trade of sparkling wine has steadily increased both in volume and in value, with an average annual growth rate of 6% and 8%, respectively (OIV, 2020a). The main factor behind this success is the off-season consumption along with a more diverse supply. This boom attracts the attention of the wine industry located in the historical wine regions, which find sparkling wine a short-term strategy to captivate new emerging consumers. Nevertheless, this strategy only makes sense if wines with special and distinctive features are produced.

Recently, there has been an increase in varietal sparkling wines resulting in cultivar-specific research combined with the use of commercially available yeasts strains (Kemp, Alexandre, Robillard, & Marchal, 2015). However, if these starter cultures show a lack of acclimatisation to the local characteristics of the area, the quality assurance and typicality of the wine could be affected, since they are not established as the biological agent responsible for fermentation. Thus, exploring the biodiversity of yeast isolated from the same viticultural areas is also recommended for the production of innovative products from low aromatic grape cultivars (Vigentini, et al., 2017). Some studies can be found in the scientific literature focused on the selection of indigenous strains (*Saccharomyces* and *non-Saccharomyces)* to be used during the first or second fermentation of sparkling wines production (Di Gianvito et al., 2018; Garofalo, Berbegal, Grieco, Tufariello, Spano, & Capozzi, 2018; Ivit, Loira, Morata, Benito, Palomero, & Suarez-Lepe, 2018; Velazquez, Zamora, Alvarez, & Ramirez, 2019).

Cava is the most produced sparkling wine in Spain and constitutes 89% of total volume manufactured in most recent years (OIV, 2020a). It is obtained by the Champenoise method using Macabeo, Xarel.lo, Parellada, Malavasía, Chardonnay, Garnacha tinta, Monastrell, Pinot Noir and Trepat as authorised grapes to produce Cavas in specific geographical areas (BOE, 2020), although the use of other grapes varieties has been also demonstrated to obtain high-quality sparkling wines (Coelho, Coimbra, Nogueira, & Rocha, 2009; Garcia, Aleixandre, Alvarez, & Lizama, 2009; Martinez-Lapuente, Guadalupe, Ayestaran, Ortega-Heras, & Perez-Magarino, 2013; Perez-Magarino, Ortega-Heras, Bueno-Herrera, Martinez-Lapuente, Guadalupe, & Ayestaran, 2015; Soares, Welke, Nicolli, Zanus, Caramao, Manfroi, et al., 2015).

Pedro Ximenez (PX) is a low aroma grape variety mostly cultivated in Montilla-Moriles wine growing region (Andalusia, Southern Spain) that is used in the production of varietal sweet wines and, along with the so-called "flor yeast" strains, responsible for the sensory characteristics of Sherry wine-types. Some papers about the use of this grape and a commercial yeast strain to elaborate sparkling wines have been recently

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published (Munoz-Redondo, Cuevas, Leon, Ramirez, Moreno-Rojas, & Ruiz-Moreno, 2017; Ruiz-Moreno, Munoz-Redondo, Cuevas, Marrufo-Curtido, Leon, Ramirez, et al., 2017). These works are mainly focused on the characterization of a part of the aroma compounds, to evaluate key markers of pre-fermentative treatments and changes caused by second fermentation or ageing on lees stages. On the other hand, the viability of flor yeasts against stress factors derived from the second fermentation has also been demonstrated (Gonzalez-Jimenez et al., 2020; Martinez-Garcia, Roldan-Romero, Moreno, Puig-Pujol, Mauricio, & Garcia-Martinez, 2020). Notwithstanding, studies based on the volatile composition and the aroma assessment of sparkling wines obtained by the joint use of PX grapes and a native *S. cerevisiae* veil-forming yeast strain, have not yet been conducted.

Within this context, the sparkling wine aroma is considered a crucial quality indicator for consumers, with sensory and volatile compounds analysis being the most widely used methods for its assessment (Hidalgo, Pueyo, Pozo-Bayon, Martinez-Rodriguez, Martin-Alvarez, & Polo, 2004; Puig-Pujol, Bertran, Garcia-Martinez, Capdevila, Minguez, & Mauricio, 2013; Vecchio, Lisanti, Caracciolo, Cembalo, Gambuti, Moio, et al., 2019). The former is the most important analysis but also costly and time-consuming; in addition to having a subjective component when it is not based on trained experts. Furthermore, given the difficulty of evaluating wines in the presence of CO_2, no internationally accepted method has been specially designed, and the methodology found in literature depends on the aims of the research (Kemp et al., 2015; White & Heymann, 2015). At the same time, the quantification of volatile compounds is revealed as necessary to establish their odorant contribution, but the biggest drawback is that information derived from their interaction is not reported. Regarding these two methodologies, most studies fail when conclusions are drawn from their individual use, or when no relationships are established from their joint use. Knowing which compounds exert major and marginal influence on sensory indicators will allow better quality control of the winemaking process of these special wines, while multivariate analysis could help to elucidate the interconnection between techniques.

Different approaches have been suggested, such as principal component analysis (PCA) (Culbert, Ristic, Ovington, Saliba, & Wilkinson, 2017; Ubeda, Kania-Zelada, del Barrio-Galan, Medel-Maraboli, Gil, & Pena-Neira, 2019), factorial or discriminant analysis (Torrens, Urpi, Riu-Aurnatell, Vichi, Lopez-Tamames, & Buxaderas, 2008; Perez-Magarino, Ortega-Heras, Martinez-Lapuente, Guadalupe & Ayestaran, 2013). Another method is partial least squares (PLS), particularly suitable for multivariate problems when the number of observations is less than the number of possible predictors (Seisonen, Vene, & Koppel, 2016). Through PLS the relationships between predictors and the response function can be inferred from weights and regression coefficients of individual predictors in most explanatory components (Herrero et al., 2016). This chemometric procedure can determine and interpret the strength of interaction through variable influence on projection (VIP) values and the influence (positive or negative) of each aroma compound on the sensory attributes by means of regression coefficients.

On the basis of these considerations, this work aims to complete previous studies based on the use of a flor yeast to elaborate PX sparkling wines and contribute to the development and implementation of instrumental technologies that complement the "test panel" in the classification of quality sparkling wines.

2. Material and methods

2.1. Chemical standards

The identification and quantification of aroma compounds were carried out with standard solutions of pure compounds of analytical grade, purchased from Sigma-Aldrich, Merck and Fluka. Pure water was obtained from a Milli-Q purification system (Millipore).

2.2. Yeast strains and experimental design

Two different *Saccharomyces cerevisiae* yeast strains were used to inoculate the wines: The *S. cerevisiae* strain P29 (CECT 11770) isolated from the Penedés grape-growing area (Spain) by the INCAVI (Institut Català de la Vinya i el Vi); the ethanol-tolerant flor yeast *S. cerevisiae* G1 strain (ATCC: MYA-2451) isolated from wines subjected to biological ageing in the Montilla-Moriles area (Southern Spain) and belonging to the Department of Microbiology collection (University of Cordoba, Spain).

2.2.1. Starter culture and yeast acclimation

Each yeast strain was incubated at 21 °C for 48 h in 500 mL of YEPD broth and, before inoculation of the base wine, yeasts were adapted to growth according to Velázquez et al. (2019) protocol (***Supplementary material 1***).

2.2.2. Base wine and fermentation conditions

The base wine (BW) was purchased from Perez Barquero cellar (Montilla-Moriles) and it was obtained by blending 80% Pedro-Ximénez, 20% Moscatel grapes from 2017 vintage; their characteristics are shown in Table 1. For the second fermentation the base wine was placed in 750-mL sparkling wine bottles, added with 24 g/L sucrose and provided with 1×10^6 cells/mL yeast population. The total group of samples (50) was divided into two batches of 25 bottles to perform the inoculation with each strain (P29 and G1 yeast strains), and then the bottles were capped with a plastic lid and metal overcap and placed in a conditioning chamber (12 ± 1 °C and 75% moisture).

The second fermentation was tracked by changes in the pressure of CO_2, measured with an external aphrometer and was considered finished when it reached 6 bar, which corresponds to the total consumption of sucrose added. At this point, samples were taken at 3, 6, 9, 12 and 15 months of ageing for each yeast (3 M, 6 M, 9 M, 12 M and 15 M, respectively). Five bottles of each condition were taken, three to perform the chemical analysis and two for sensory analysis (***Supplementary Fig. 1***).

2.3. Oenological parameters

The International Methods of Analysis of Wines and Musts (OIV, 2020b) were used to analyse the ethanol, sugar content, pH, total and volatile acidity. Malic acid and lactic acid were measured by a reflectometric test (Reflectoquant, Millipore Sigma, Germany) and the yeast assimilable nitrogen (YAN) by the method described by Shively and Henick-Kling (2001). Finally, the total phenol index (TPI) considered as A_{280}, and the chromatic parameters A_{420}, A_{520}, A_{620} were evaluated in a DU-640 spectrophotometer (Beckman–Coulter, Brea, CA). Each biological replication was analysed twice.

2.4. Volatilome analysis

The wine volatilome is constituted by major volatile compounds (≥10 mg/L) and minor volatile compounds (<10 mg/L), classified according to their contents.

2.4.1. Major volatile compounds and polyols

An Agilent 6890 gas chromatograph fitted with a CP-WAX 57 CB capillary column (60 m, 0.25 mm internal diameter, 0.4 μm film thickness; Varian, Palo Alto, CA) and provided with a flame ionization detector (FID), using the chromatographic conditions previously described by Martinez-Garcia, Garcia-Martinez, Puig-Pujol, Mauricio, and Moreno (2017). The method consists of a direct injection of 1 μL of the sparkling wine sample spiked with an internal standard solution of 4-methyl-2-pentanol at 1 g/L. Confirmation of the identified compounds was performed, under the same chromatographic conditions described, using an Agilent 7890A GC coupled to a MSD5975C Mass Detector from

Table 1
Composition of base wine (BW) and the sparkling wines obtained for both yeast strains at different ageing times.

Parameter	P29						G1					Y	t	Yxt
	BW	3 M	6 M	9 M	12 M	15 M	3 M	6 M	9 M	12 M	15 M	p	p	p
Ethanol (% v/v)	11.45^{a} ± 0.05	12.45^{cde} ± 0.00	12.38^{bcde} ± 0.10	12.47^{de} ± 0.08	12.30^{b} ± 0.05	12.43^{bcde} ± 0.10	12.32^{bc} ± 0.10	12.42^{bcde} ± 0.08	12.50^{e} ± 0.10	12.35^{bcd} ± 0.05	12.47^{de} ± 0.13	ns	ns	ns
Reducing sugars (g/L)	1^{c} ± 0	0.5^{b} ± 0.2	0.6^{b} ± 0.2	0.5^{b} ± 0.2	0.2^{a} ± 0.0	0.4^{b} ± 0.0	0.6^{b} ± 0.2	0.6^{b} ± 0.2	0.5^{b} ± 0.2	0.2^{a} ± 0.0	0.3^{a} ± 0.1	ns	***	ns
Propane-1,2,3-triol (glycerol) (g/L)	3.19^{b} ± 0.05	2.4^{a} ± 0.1	8.0^{d} ± 0.1	9^{d} ± 1	5.9^{c} ± 0.2	5.8^{c} ± 0.2	3.0^{b} ± 0.1	8.3^{d} ± 0.3	8.3^{d} ± 0.3	7.7^{d} ± 0.3	5.4^{c} ± 0.4	ns	*	ns
Volatile acidity (g/L)	0.31^{bc} ± 0.02	0.29^{abc} ± 0.02	0.36^{c} ± 0.09	0.45^{d} ± 0.06	0.35^{c} ± 0.05	0.26^{ab} ± 0.06	0.30^{abc} ± 0.03	0.24^{a} ± 0.02	0.34^{c} ± 0.02	0.33 ^{cd} ± 0.03	0.35^{c} ± 0.02	ns	*	ns
Total acidity (g/L)	5.06^{d} ± 0.00	5.28^{e} ± 0.04	4.97^{bcd} ± 0.12	5.08^{de} ± 0.04	4.90^{b} ± 0.04	4.95^{bcde} ± 0.08	5.03^{bcd} ± 0.00	4.94^{bc} ± 0.07	5.10^{d} ± 0.07	5.03^{bcd} ± 0.00	4.68^{a} ± 0.22	ns	ns	ns
pH	3.16^{d} ± 0.01	3.09^{abc} ± 0.05	3.06^{a} ± 0.04	3.11^{c} ± 0.01	3.11^{c} ± 0.01	3.16^{d} ± 0.01	3.07^{a} ± 0.01	3.08^{ab} ± 0.01	3.06^{a} ± 0.01	3.08^{abc} ± 0.00	3.17^{d} ± 0.00	ns	**	ns
Malic acid (g/L)	1.31^{e} ± 0.00	1.08 ^{cd} ± 0.03	1.25^{de} ± 0.04	0.91^{bc} ± 0.14	0.86^{b} ± 0.08	1.00^{bc} ± 0.04	1.01^{bc} ± 0.03	1.01^{bc} ± 0.11	0.55^{a} ± 0.14	0.94^{bc} ± 0.10	1.06 ^{cd} ± 0.17	ns	**	ns
Lactic acid (g/L)	0.15^{c} ± 0.00	0.13^{c} ± 0.03	0.16^{c} ± 0.01	0.16^{b} ± 0.08	0.0032^{a} ± 0.0002	0.17^{d} ± 0.04	0.30^{b} ± 0.04	0.13^{c} ± 0.02	0.07^{b} ± 0.02	0.0031^{a} ± 0.0001	0.15^{c} ± 0.04	ns	*	ns
TPI (AU 280 nm)	7.96^{d} ± 0.02	7.6 ^{cd} ± 0.1	7.7 ^{cd} ± 0.1	7.3^{c} ± 0.5	4.7^{a} ± 0.4	7.4^{c} ± 0.3	7.3^{c} ± 0.2	7.6 ^{cd} ± 0.1	7.2^{c} ± 0.3	5.9^{b} ± 0.2	7.3^{c} ± 0.2	ns	*	ns
A_{420} (AU 420 nm)	0.131^{b} ± 0.001	0.35^{d} ± 0.02	0.48^{e} ± 0.05	0.29^{c} ± 0.03	0.11^{a} ± 0.01	0.098^{a} ± 0.004	0.10^{a} ± 0.02	0.13^{b} ± 0.03	0.15^{b} ± 0.02	0.09^{a} ± 0.01	0.100^{a} ± 0.001	***	***	***
A_{520} (AU 520 nm)	0.046^{c} ± 0.001	0.26^{e} ± 0.02	0.36^{f} ± 0.04	0.21^{e} ± 0.04	0.03^{b} ± 0.01	0.020^{a} ± 0.002	0.03^{b} ± 0.01	0.06 ^{cd} ± 0.02	0.07^{d} ± 0.01	0.022^{a} ± 0.003	0.021^{a} ± 0.001	***	***	*
A_{620} (AU 620 nm)	0.020^{b} ± 0.001	0.23^{de} ± 0.02	0.34^{e} ± 0.03	0.19^{d} ± 0.04	0.02^{b} ± 0.01	0.006^{a} ± 0.001	0.02^{b} ± 0.01	0.06^{c} ± 0.02	0.06^{c} ± 0.01	0.010^{a} ± 0.002	0.0075^{a} ± 0.0004	**	***	ns
Colour intensity (A_{420} + A_{520} + A_{620})	0.197^{c} ± 0.003	0.8^{e} ± 0.1	1.18^{f} ± 0.1	0.7^{e} ± 0.1	0.16^{bc} ± 0.02	0.125^{a} ± 0.008	0.16^{ab} ± 0.04	0.3^{d} ± 0.1	0.29^{d} ± 0.04	0.127^{ab} ± 0.011	0.13^{ab} ± 0.002	***	***	**
Tonality (A_{420}/A_{520})	2.84^{c} ± 0.4	1.38^{a} ± 0.03	1.35^{a} ± 0.04	1.4^{a} ± 0.2	3.3^{c} ± 0.4	4.9^{d} ± 0.3	4^{c} ± 1	2.2^{b} ± 0.4	2.1^{b} ± 0.2	4.4^{d} ± 0.5	4.8^{d} ± 0.1	***	**	ns
YAN (mg/L)	63^{a} ± 0	96^{ef} ± 4	98^{f} ± 0	84^{cde} ± 14	98^{f} ± 0	79 ^{cd} ± 8	79 ^{cd} ± 8	79 ^{cd} ± 8	75^{bc} ± 8	89^{def} ± 8	65^{ab} ± 8	**	ns	ns

a. b. c. e 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 test. ns - non-significant. *, **, *** indicate significance at 0.05, 0.01 and 0.001 level, respectively. Identification of wine samples: BW: base wine. 3M: sparkling wines with 3 months of ageing. 6M: sparkling wines with 6 months of ageing. 9M: sparkling wines with 9months of ageing. 12M: sparkling wines with 12 months of ageing. 15M: sparkling wines with 15 months of ageing. P29: *S. cerevisiae P29 strain.* G1: *S. cerevisiae G1 strain*. TPI: total phenol index. YAN: yeast assimilable nitrogen. *Y:* yeast factor. *t:* time factor. *Yxt:* interaction.

Agilent Technologies (Wilmington, DE,).

2.4.2. Minor volatile compounds

A hyphenated technology was used to analyse this fraction that consisted of a multipurpose sampler (MPS), and a thermal desorption unit (TDU) from Gerstel GmbH (Mülheim an der Rühr, Germany) coupled to an Agilent GC7890A–MSD 5975GC system. The GC was fitted with an HP-5MS capillary column (30 m χ0.25 mm i.d. χ 0.25 μm film thickness) and the operating conditions for GC and MSD were described by Lopez de Lerma, Peinado, Puig-Pujol, Mauricio, Moreno, and Garcia-Martinez (2018). Before GC–MS analysis, sparkling wine samples were subjected to stir bar sorptive extraction (SBSE), which consisted of their stirring (1200 rpm for 100 min, 20 °C) with a Twister (0.5 mm film thickness and 10 mm length) coated with polydimethylsiloxane (PDMS), accordingly to Martinez-Garcia et al. (2017). The analysis was performed in triplicate for each biological replicate. The quantification and identification criteria were previously described by Martinez-Garcia, et al. (2021) and a detailed information is in ***Supplementary Material 2***.

2.4.3. Calculation of odorant series, constitutive and discriminant compounds

The contribution of each volatile compound to the wine aroma can be qualitatively assessed by its odour descriptor and quantitatively by its odour activity value (OAV), which is defined as the ratio concentration/odour perception threshold (OPT). Hence, those compounds with the same or similar descriptor can be considered jointly, constituting one odorant series (OS), whose OAV is calculated by adding the OAV of the compounds it contains. The OAV calculation allows an objective evaluation of aroma intensity for each compound, because of the impossibility of decoupling the interactions with other wine volatiles (Martínez-García, et al., 2021). The ratios OAVmax/OAVmin were also calculated to classify a compound as constitutive (OAVmax > 1 and 1 < OAVmax/OAVmin < 2) and as discriminant (OAVmax > 1 and OAVmax/OAVmin > 2); based on the procedure described by Herrero et al. (2016). Only quantified compounds were used to calculate OAVs, being excluded those without a commercially available standard or without OPT available in the wine literature.

2.5. Sensorial analysis

Sparkling wines were subjected to the judgment of 12 expert tasters under the conditions described in ISO (2014), in order to establish an organoleptic profile and the differences due to the yeast strain and ageing time. The five sampling points (3 M, 6 M, 9 M, 12 M, 15 M) were analysed twice in 10 tasting sessions, three sensory tests being carried out (one differential and two descriptive tests).

The differential test was a triangle test (*t*-test) based on ISO (2004). Randomised samples of 25 to 30 mL were served at 6 to 8 °C in clear glasses labelled with random numbers and covered with petri dishes to preserve aroma. In each series, two samples were identical (the same yeast strain) and one different, the taster being asked to distinguish the latter. Secondly, a descriptive test (DA1) was done to evaluate generic visual, olfactory and taste attributes with the tasting card recommended by OIV (OIV, 2009). Samples were presented in a blind randomised sequence and the eleven sensory attributes were immediately assessed and rated by panellists (lapse time between pouring and serving < 30 s). The sensory attributes were limpidity (1–5), effervescence (2–10), visual aspect (2–10), aroma intensity (3–7), aroma quality (6–14), characteristic aroma (3–7), taste intensity (3–7), taste quality (6–14), persistence (3–7), characteristic taste (3–7) and harmony (8–12). The scores sum allows classification of the wines as rejected (0–7), inadequate (8–23), average (24–44), better than average (45–62), good (63–78), very good (79–90) and excellent (>90). In term of visual aspects, special attention was paid not only to colour but also foam characteristics. Wines were tasted in triplicate and the final score (average of each judge) was subjected to statistical analysis. The third sensory analysis consisted of a descriptive test (DA2) designed to evaluate 8 aroma descriptors (chemical, fruity, floral, fatty, balsamic, vegetable, empyreumatic and spicy), selected by consensus with respect to previous results for these yeast strains (Martinez-Garcia et al., 2017, 2020, 2021) and the protocol presented in ***Supplementary Material 3***. The odour intensity for each descriptor was evaluated using an unstructured 10-cm line scale anchored on the left with "low" intensity and on the right with "high" intensity that was converted to a 9-point scale with an Excel spreadsheet. For this test, the same group of samples subjected to the chemical analysis were used. Thus, at the time of sampling for chemical analysis, a volume of 300–350 mL was allocated from each bottle and kept at 12 °C overnight until the tasting session. Given the small sample volume available for this test, each bottle from the triplicate was only evaluated by 4 panellists, the mean value being used to build predictive models.

To avoid changes in sensory perception by CO_2 during sensory tests, this gas was removed from wine samples by partial vacuum using a Venturi pump system for 1 min.

2.6. Data statistical analysis

All data showed are the average values of three biological replicates, analysed for each studied condition and previously pre-processed (autoscaling and centring). Univariate analysis (ANOVA) was performed through Statgraphics Plus version 2 software from STSC, Inc. (Rockville, MD). General parameters and volatile aroma compounds were processed using two-way analysis of variance and least significant test (LSD Fisher's test) to study the individual effect of yeast and ageing, while multivariate analysis of variance (MANOVA) established their interaction. Differences at $p < 0.05$ were considered to be statistically significant.

A cross-platform integrated development environment for the statistical language (Rstudio) was used to perform multivariate analysis. Unsupervised pattern recognition techniques, such as heatmap and principal component analysis (PCA), were applied to establish differences related to the two factors studied. Finally, partial least squares (PLS) regression was used as the supervised recognition technique to discern the most influential key markers in the wines' sensory profiles. For this, PLS models were built from different datasets (volatile fraction and sensory analysis) previously log10-transformed and scaled.

3. Results and discussion

3.1. Evolution of endogenous CO_2 pressure

Supplementary Fig. 2 shows the pressure evolution of endogenous CO_2 gas generated by both yeasts during the second fermentation, revealing that P29 yeast strain had a faster CO_2 production kinetic than G1 yeast strain, since the time it takes to reach 6 bars is shorter (58 and 71 days, respectively). This is explained by the greater effect that CO_2 overpressure has on the G1 cells viability, while the difference observed in fermentation kinetics when comparing with previous works is a consequence of the base wine composition and the environmental conditions used for fermentation (Martínez-García et al., 2017, 2020; Vigentini et al., 2017).

3.2. Oenological features

Table 1 shows the mean values and standard deviation for the base wine (BW) and the sparkling wines obtained by P29 and G1 strains at different ageing time (3 M, 6 M, 9 M, 12 M and 15 M). In addition, the significance levels obtained by a two-way ANOVA are also shown to stablish differences between sparkling wines in terms of yeast (*Y*), time (*t*) and their interaction (*Yxt*). To perform the analysis, sparkling wines obtained at 3 and 6 months were considered as young sparkling wines, while the remaining wines (9, 12 and 15 months) were considered as aged sparkling wines. To begin with, all the obtained wines fulfilled the legal and quality standards established in the Spanish normative (BOE, 2020), showing contents in volatile acidity and glycerol suitable for high quality sparkling wines (Vigentini et al., 2017). Regarding the two factors studied, only colour intensity (CI), tonality and absorbance values showed dependence on both. During the first months of ageing, these parameters and the total phenol index (TPI) show an increase when compared to BW, followed by a decrease, in line with the results of Ubeda et al. (2019). These changes are respectively a consequence of furanic compounds derived from the added sugar to initiate the second fermentation and the adsorption of polyphenols on yeast lees or their own polymerization and precipitation during ageing. Furthermore, aged sparkling wines also displayed higher values in pH, volatile acidity and lower values in reducing sugars, malic acid, lactic acid. Nevertheless, only changes for pH were described in the literature during the same ageing period (Ubeda et al., 2019), while the decrease in glycerol content with ageing can be explained by adsorption phenomena or by its possible combination with aldehydes and acids to form acetals and esters, respectively. In contrast, yeast assimilable nitrogen (YAN) was only dependent on yeast strain, being higher for P29 wines. Finally, the ethanol content and total acidity did not show dependence with the considered factors.

3.3. Wine volatilome

Supplementary Table 1 shows the CAS number, LRI values, odour descriptors, perception thresholds (OPT) and the odorant series (OS) for 82 metabolites identified in this work. All of them are considered wine volatiles, exception made for the polyols 2,3-butanediol and glycerol, which are semi-volatile and non-volatile compounds, respectively. Based on their chemical structure, there are identified: 8 alcohols, 6 aldehydes, 5 ketones, 7 carboxylic acids, 25 esters, 5 lactones, 8 terpenoids, 3 norisoprenoids, 7 volatile phenols, 5 furanic compounds and 2 polyols. Esters were also grouped 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), higher alcohols acetates (HAAs) and miscellaneous.

A Venn diagram was generated to illustrate the number of compounds identified in the wine samples (*Supplementary* Fig. 3). Each

condition is represented by a circle whose unions and intersections show the number of common compounds for two or three conditions and those specific for each one. According to this diagram, there is a decrease in the total number of common compounds determined in the BW and the two-yeasts wines, as ageing time goes on. This decrease is also observed in the number of common compounds identified among P29 and G1 sparkling wines after 6 months of ageing (*Supplementary* Fig. 3-*B*). The differences found between the first and second fermentation have been previously described and are attributed to the ability of the yeast strain to produce aromas during bottle fermentation (Torrens et al., 2008; Welke, Zanus, Lazzarotto, Pulgati, & Zini, 2014; Martínez-García et al., 2017, 2020).

Although individual compound comparisons cannot be made, from a quantitative point of view (Table 2) the total content observed for each chemical family is similar to the content shown in previous studies carried out with P29 yeast strain to produce multivarietal Cava sparkling wines aged 15 and 24 months (Martínez-García et al., 2021). Differences observed in terpenoid and norisoprenoid families are linked to the grape varieties used and their own adaptation to the edaphoclimatic conditions of the growing area (Coelho et al., 2009; Munoz-Redondo, Ruiz-Moreno, Puertas, Cantos-Villar, & Moreno-Rojas, 2020).

3.3.1. Effect of yeast strain and ageing time on wine volatilome

Table 2 shows the volatilome dataset, grouped by chemical families, and the statistical significance level obtained by two-way ANOVA, regarding the two factors (*Y, t)* and their interaction (*Yxt*). As in the general parameters, to carry out the analysis, young wines (3 and 6 months) were considered as a group, while the rest (9, 12 and 15 months) were considered the group of aged sparkling wines.

According to this table, both factors affect the group of norisoprenoids content, while only ageing induces an increase in the content of ketones and a decrease in the content of alcohols, acids, volatile phenols, furanic compounds and the total esters (EBBAs, HAAs, IEFAs and miscellaneous, mainly). Pozo-Bayon et al. (2003), stated that ageing is the greatest contributor to changes in the volatile fraction of sparkling wines, regardless of the yeast strain and the 'tirage' solution used. Despite this, in the current study some specific compounds showed dependence on both factors.

The alcohols family depends significantly on ageing, showing a decrease in isobutanol (**3**), isoamyl alcohols (**4**), 2-phenyl-1-ethanol (**5**) and *trans*-2-hexen-1-ol (**14**), while methanol (1) increases its content when comparing young wines with aged sparkling wines (Table 2). This dependence with ageing has also been observed in previous studies for compounds 3 and 4 (Martínez-García et al., 2021). The importance of higher alcohols lies in their influence on the quality of wines, their content being directly correlated to the yeast metabolism (Garofalo et al., 2018). However, in the current study only *trans*-2-hexen-1-ol (**14**), 1-hexanol (**15**) and 2-ethyl-1-hexanol (**16**) are dependent on yeast factor, some of which have already been mentioned by Torrens et al., (2008). Concerning aldehydes and ketones, only benzaldehyde (**18**) showed dependence with yeast strain, contrary to the results obtained by Torrens et al., (2008). On the other hand, decanal (**21**) and acetoin (**7**) showed dependence with ageing, the former not being identified in the older wines (9, 12 and 15 months) and the latter in 3 M−aged wines. No changes for these compounds were described in the literature with ageing.

Aged wines showed a respective decrease of 83% and 94% for the total content in acids and esters. High hydrophobicity and thermodynamic instability of compounds, such as acids and esters, are the main reason for their decrease with ageing, since their adsorption on yeast lees takes place (Gallardo-Chacon, Vichi, Lopez-Tamames, & Buxaderas, 2010). Di Gianvito et al. (2018) attributed the decrease in esters during wine ageing to the release of hydrolytic enzymes as well as the limitation in the availability of precursors such as octanoic acid. In the current study, the greatest contributors for this trend were octanoic acid (**27**) and some compounds of EEFAs group, such as ethyl lactate (**9**), ethyl butanoate (**34**), ethyl 3-hydroxyoctanoate (**33**), ethyl tetradecanoate (**41**) and ethyl hexadecanoate (**42**). Compounds like ethyl 3-methylbutanoate (**44**), ethyl 2-methyloctanoate (**45**), isoamyl acetate (**52**), 2-phenylethyl acetate (**50**) and 2-phenylethyl hexanoate (**54**), also showed a decrease between young and aged sparkling wines, ranging from 0 μg/L to 189 μg/L. This decreasing trend is also observed for compounds **50** and **52** in 24-months aged sparkling wines (Martínez-García et al., 2021). In contrast, Ruiz-Moreno et al., (2017) described an increase in the content of HAAs and EBBAs as a consequence of the interaction effect between skin maceration and ageing factor for PX sparkling wines. Furthermore, ethyl 9-decenoate (**38**), ethyl decanoate (**39**), ethyl dodecanoate (**40**) and isoamyl octanoate (**53**) showed significant differences with yeast factor, with G1 wines having a percentage decrease that ranged from 19 to 64%, compared to P29. Some authors attribute the higher content of esters in wines to the strong autolytic ability of the strain and also to their different esterase activity (Di Gianvito et al., 2018). In contrast, ethyl acetate (**8**), diethyl succinate (**10**) and isobornyl acetate (**54**) with ageing and ethyl propanoate (**33**) for aged G1 wines, showed the opposite trend. No changes were observed in the group of MEFAs regarding these two factors, while intramolecular esters, such as caprolactone (**57**), were only detected in BW and G1 wines aged until 3–6 months. These wines, along with those from P29 at the same ageing time, also registered higher content in γ-nonalactone (**58**), while γ-butyrolactone (**56**) was only quantified in wines from the two yeasts at 9 months and in those of P29 at 12 months.

The varietal compounds belonging to terpenoids (**63**, **64**, and **66**) and norisoprenoids (**68**, **69**, **70**) exhibited a general decrease with ageing, while neroloxide (**65**) shows the opposite trend. The decrease in terpenoids is also observed by Soares et al., (2015) when monitoring the second fermentation at 0, 6, 12 and 20 days. Conversely, only the contents of *o*-cymene (**60**) and β-damascenone (**69**) are significantly dependent on yeast factor, the latter being in agreement with the results of Torrens et al., 2008. These authors attribute the changes observed for C6 alcohols and terpinols with ageing to their interaction with yeast mannoproteins or their enzymatic activity when they are glycosylated.

Finally, some volatile phenols are affected by ageing (**72**, **75**), the yeast strain (**77**) or both (**74**), with older wines having the lowest content in them, as well as in the furanic compounds **79** and **82**. The importance of these compounds lies in their contribution to flavour, colour and smell, which explains the results observed **in** Table 1 for colour intensity and A_{280}.

3.3.2. Volatilome heat map

The heat map in ***Supplementary Fig. 4*** provides a complete overview about the effect of the two factors studied on the content of each compound tested. The square Euclidean distance and Ward's method were used as grouping rule and as a measure of the proximity among samples. Based on this figure, compounds (in columns) were grouped in 8 clusters and their relative contents coloured in blue and red (low and high levels, respectively). Thus, cluster 1 has a greater number of terpenoids and EEFAs, some of which exceed OAV > 1 (**8**, **35**, **36** and **39**). The 9 M wines are the most representative for this group contributing to all odorant series, except to vegetal (***Supplementary*** Table 1). Pérez-Magariño et al. (2015) also found a higher content in EEFAs and lower content in HAAs at 9 months when compared with 18 and 30 months of ageing. The compounds belonging to cluster 2 are mainly alcohols, the largest contributors to chemical series. However, their content is<400 mg/L (Table 2), which is considered a positive contribution to the wine aroma (Martínez-García et al., 2017). This group had the lowest amount for the 15 M wines and the highest level for the base wine (BW) and the wines subjected to short ageing time (P29-3 M and G1-3 M). From them, compounds **4**, **5** and **6** showed OAV > 1, despite having less content than those previously published with these yeasts (Martínez-García et al., 2020, 2021). A similar trend is observed for these wine samples and seventeen compounds in cluster 3 (3 alcohols, 1 aldehyde, 3 acids, 3 volatile phenols, 6 esters and 1 terpene); eight of which exceed their OPT

Table 2
Average concentrations ± standard deviation of major and minor aroma compounds (mg/L, except where indicated) in base wine (BW) and the sparkling wines.

			P29					*G1*					***Y***	***t***	***Yxt***
N°	Compound	BW	3 M	6 M	9 M	12 M	15 M	3 M	6 M	9 M	12 M	15 M	***p***	***p***	***p***
	Σ Alcohols	365^{g} ± 11	360^{fg} ± 7	359^{efg} ± 9	361^{fg} ± 6	345^{de} ± 16	282^{b} ± 3	369^{g} ± 5	328^{c} ± 7	348^{def} ± 5	342^{cd} ± 7	269^{a} ± 9	ns	*	ns
1	Methanol	56^{d} ± 1	50^{b} ± 1	60^{e} ± 2	65^{f} ± 2	56^{d} ± 1	60^{e} ± 1	51^{bc} ± 1	42.8^{a} ± 0.4	53^{c} ± 3	56^{d} ± 1	70^{g} ± 4	ns	**	ns
2	1-Propanol	50^{fg} ± 3	51^{g} ± 1	45^{bcd} ± 4	49^{efg} ± 2	47^{def} ± 4	42^{ab} ± 1	51^{fg} ± 1	43^{bc} ± 1	46^{cde} ± 1	47^{defg} ± 2	39^{a} ± 2	ns	ns	ns
3	2-Methyl-1-propanol (isobutanol)	18^{cd} ± 1	18.5^{d} ± 0.3	19^{d} ± 1	19^{d} ± 1	19^{d} ± 1	13.9^{b} ± 0.3	18.3^{cd} ± 0.3	18.2^{cd} ± 0.3	18.3^{cd} ± 0.3	17^{c} ± 1	12^{a} ± 1	ns	*	ns
4	Isoamyl alcohols†	**214^{de} ± 7**	**220^{ef} ± 6**	**212^{cde} ± 7**	**210^{cd} ± 2**	**203^{c} ± 11**	**149^{b} ± 1**	**228^{f} ± 3**	**207^{cd} ± 4**	**211^{cde} ± 3**	**204^{cd} ± 3**	**135^{a} ± 7**	ns	**	ns
5	2-Phenyl-1-ethanol	**18.3^{fg} ± 0.3**	**19^{g} ± 1**	**17.7^{ef} ± 0.2**	**16.5^{cd} ± 0.2**	**16.8^{cde} ± 0.4**	**14.8^{b} ± 0.4**	**19^{g} ± 1**	**16^{c} ± 1**	**17.0^{de} ± 0.4**	**14.6^{b} ± 0.4**	**13^{a} ± 1**	ns	***	ns
14	*trans*-2-Hexen-1-ol	**4.7^{h} ± 0.1**	**3.3^{f} ± 0.2**	**2.3^{e} ± 0.1**	**2.3^{e} ± 0.3**	**1.7^{c} ± 0.1**	**1.37^{b} ± 0.02**	**2.6^{g} ± 0.2**	**1.67^{c} ± 0.04**	**1.9^{d} ± 0.2**	**1.34^{b} ± 0.02**	**0.9^{a} ± 0.1**	*	**	ns
15	1-Hexanol	**3.3^{d} ± 0.2**	n.f.^{a}	**2.1^{bc} ± 0.1**	n.f.^{a}	**2^{b} ± 1**	2.1^{c} ± 0.1	n.f^{a}.	n.f.^{a}	n.f.^{a}	1.95^{bc} ± 0.03	n.f^{a}	*	ns	ns
16	2-Ethyl-1-hexanol (μg/L)	1202^{e} ± 48	n.f.^{a}	n.f.^{a}	44^{b} ± 3	n.f.^{a}	n.f.^{a}	n.f.^{a}	335^{c} ± 2	832^{d} ± 27	n.f.^{a}	n.f	*	ns	ns
	Σ Aldehydes	106^{g} ± 3	83^{def} ± 1	84^{ef} ± 2	91^{f} ± 4	88^{fg} ± 4	64^{b} ± 2	75^{c} ± 3	78^{cd} ± 2	81^{def} ± 2	87^{efg} ± 2	55^{a} ± 5	ns	ns	ns
6	Acetaldehyde	**106^{h} ± 3**	**83^{def} ± 1**	**84^{ef} ± 2**	**91^{g} ± 4**	**88^{fg} ± 4**	**64^{b} ± 2**	**75^{c} ± 3**	**78^{cd} ± 2**	**81^{def} ± 2**	**87^{efg} ± 2**	**55^{a} ± 5**	ns	ns	ns
17	Heptanal (μg/L)	1.6^{b} ± 0.2	n.f.^{a}	n.f.^{a}	n.f.^{a}	n.f.^{a}	n.f.^{a}	n.f.^{a}	n.f.^{a}	n.f.^{a}	n.f.^{a}	n.f.^{a}	ns	ns	ns
18	Benzaldehyde (μg/L)	84^{g} ± 8	46^{e} ± 4	22^{b} ± 2	62^{f} ± 8	66^{f} ± 6	31^{d} ± 1	24^{bc} ± 4	16.8^{a} ± 0.3	29^{cd} ± 1	16^{a} ± 4	39^{e} ± 2	***	ns	ns
19	Octanal (μg/L)	0.7^{b} ± 0.1	3.7^{e} ± 0.3	n.f.^{a}	n.f.^{a}	n.f.^{a}	4.3^{f} ± 0.2	0.9^{c} ± 0.1	n.f.^{a}	n.f.^{a}	n.f.^{a}	3.1^{d} ± 0.2	ns	ns	ns
20	Nonanal (μg/L)	**24^{f} ± 1**	**30^{g} ± 2**	n.f.^{a}	n.f.^{a}	8^{c} ± 1	6^{b} ± 1	**29^{g} ± 1**	n.f.^{a}	n.f.^{a}	9^{d} ± 1	**11.4^{e} ± 0.4**	ns	ns	ns
21	Decanal (μg/L)	1.6^{b} ± 0.4	**8^{d} ± 1**	2^{b} ± 1	n.f.^{a}	n.f.^{a}	n.f.^{a}	3.2^{c} ± 0.1	2.9^{c} ± 0.4	n.f.^{a}	n.f.^{a}	n.f.^{a}	ns	***	ns
	Σ Ketones	1.3^{a} ± 0.1	4.0^{d} ± 0.1	6.1^{e} ± 0.2	6.9^{f} ± 0.1	8.4^{g} ± 0.2	8.4^{g} ± 0.4	3.4^{b} ± 0.1	6.8^{f} ± 0.5	3.7^{c} ± 0.1	8.4^{g} ± 0.3	8.9^{g} ± 0.3	ns	**	ns
7	Acetoin	n.f.^{a}	n.f.^{a}	4.8^{cd} ± 0.2	6.7^{g} ± 0.1	4.4^{c} ± 0.2	5.0^{de} ± 0.4	n.f.^{a}	4.8^{de} ± 0.3	3.6^{b} ± 0.1	5.5^{f} ± 0.4	5.1^{ef} ± 0.2	ns	***	ns
22	1,2-Cyclopentanedione[1]	n.f.^{a}	2.2^{e} ± 0.1	1.34^{b} ± 0.02	n.f.^{a}	1.8^{c} ± 0.1	2.2^{e} ± 0.1	1.8^{c} ± 0.1	2.0^{d} ± 0.2	n.f.^{a}	1.4^{b} ± 0.1	2.5^{f} ± 0.1	ns	ns	ns
23	6-Methyl-5-hepten-2-one (μg/L)[2]	n.f.^{a}	12^{g} ± 2	4.9^{c} ± 0.3	3^{b} ± 1	11^{fg} ± 2	9^{ef} ± 1	7.6^{de} ± 0.4	9^{ef} ± 1	5^{c} ± 1	7.0^{d} ± 0.3	10^{fg} ± 2	ns	ns	ns
24	3-Methyl-1,2-cyclopentanedione (μg/L)[3]	22^{g} ± 2	13^{de} ± 1	10^{c} ± 1	7^{b} ± 1	12^{d} ± 1	14^{ef} ± 1	11^{c} ± 1	20^{g} ± 2	n.f.^{a}	10.2^{c} ± 0.2	15^{f} ± 1	ns	ns	ns
25	Benzophenone[4]	1.3^{e} ± 0.1	1.7^{h} ± 0.1	n.f.^{a}	0.176^{b} ± 0.004	2.2^{i} ± 0.2	1.17^{d} ± 0.01	1.60^{g} ± 0.02	n.f.^{a}	0.19^{c} ± 0.01	1.5^{f} ± 0.1	1.18^{d} ± 0.04	ns	ns	ns
	Σ Acids	156^{g} ± 23	77^{f} ± 4	76^{f} ± 1	13^{c} ± 1	8.8^{b} ± 0.2	32^{d} ± 1	66^{e} ± 6	73^{ef} ± 1	31^{d} ± 5	4.1^{a} ± 0.3	34^{d} ± 3	ns	***	ns
26	Butanoic acid	0.65^{f} ± 0.02	0.3^{e} ± 0.1	0.10^{c} ± 0.02	0.010^{b} ± 0.001	n.f.^{a}	n.f.^{a}	0.34^{e} ± 0.02	0.19^{d} ± 0.01	0.018^{c} ± 0.001	n.f.^{a}	n.f.^{a}	ns	***	ns
27	Octanoic acid	**139^{g} ± 22**	**68^{f} ± 3**	**66^{f} ± 1**	**12^{c} ± 1**	**7.6^{b} ± 0.2**	**31^{de} ± 1**	**60^{f} ± 5**	**62^{f} ± 1**	**27^{d} ± 4**	**2.7^{a} ± 0.3**	**33^{e} ± 3**	ns	***	ns
28	Decanoic acid	**15^{g} ± 1**	**7^{f} ± 2**	**6.3^{f} ± 0.1**	n.f.^{a}	0.0791^{b} ± 0.0003	n.f.^{a}	**4.1^{d} ± 0.4**	**5.3^{e} ± 0.2**	**2.85^{c} ± 0.4**	n.f.^{a}	0.082^{b} ± 0.002	ns	***	*
29	Dodecanoic acid	n.f.^{a}	0.28^{c} ± 0.01	0.49^{e} ± 0.04	n.f.^{a}	n.f.^{a}	0.152^{b} ± 0.001	n.f.^{a}	0.34^{d} ± 0.01	n.f.^{a}	n.f.^{a}	n.f.^{a}	ns	***	ns
30	Tetradecanoic acid (μg/L)	822^{a} ± 0	964^{d} ± 5	1041.74^{e} ± 0.03	822^{a} ± 0	822^{a} ± 0	959^{cd} ± 28	899^{b} ± 9	1103^{f} ± 10	938^{cd} ± 54	962^{d} ± 3	925^{bc} ± 1	ns	***	ns
31	Hexadecanoic acid (μg/L)	811^{f} ± 2	612^{e} ± 77	2058^{g} ± 185	405^{bcd} ± 29	206^{a} ± 21	428^{cd} ± 4	448^{d} ± 55	3276^{h} ± 120	387^{bc} ± 37	404^{bcd} ± 16	368^{b} ± 30	ns	***	ns
32	Octadecanoic acid (μg/L)[5]	262^{de} ± 17	282^{e} ± 22	461^{f} ± 34	126^{b} ± 1	54^{a} ± 0	254^{d} ± 3	212^{c} ± 19	429^{f} ± 9	196^{c} ± 9	54^{a} ± 0	54^{a} ± 0.00	ns	***	ns
	Σ *EEFAs*	74^{abc} ± 4	78^{de} ± 1	76^{cd} ± 1	83^{f} ± 2	71^{ab} ± 2	75^{bcd} ± 1	81^{ef} ± 3	77^{cde} ± 3	77^{cde} ± 1	76^{cd} ± 1	70^{a} ± 5	ns	ns	ns

(*continued on next page*)

Table 2 (*continued*)

N°	Compound	BW	*P29* 3 M	*P29* 6 M	*P29* 9 M	*P29* 12 M	*P29* 15 M	*G1* 3 M	*G1* 6 M	*G1* 9 M	*G1* 12 M	*G1* 15 M	***Y*** *p*	***t*** *p*	***Yxt*** *p*
8	Ethyl acetate	**28^{a} ± 1**	**31^{b} ± 1**	**36^{de} ± 1**	**40^{f} ± 1**	**34 ^{cd} ± 1**	**35.6^{cde} ± 0.1**	**33^{bc} ± 2**	**35^{cde} ± 2**	**36^{cde} ± 1**	**37^{ef} ± 1**	**33^{bc} ± 3**	ns	*	ns
9	Ethyl lactate	27.3 ^{g} ± 0.4	26 ^{g} ± 1	17.9^{de} ± 0.3	19.7^{f} ± 0.3	16.6^{c} ± 0.4	12^{b} ± 1	26 ^{g} ± 1	18 ^{cd} ± 1	19^{ef} ± 1	18.5^{def} ± 0.2	10^{a} ± 1	ns	**	ns
10	Diethyl succinate	16 ^{cd} ± 4	12.1^{a} ± 0.3	14.1^{bcd} ± 0.2	15.1^{bcd} ± 0.4	13.7^{abc} ± 0.4	20^{e} ± 1	13.9^{abc} ± 0.3	16.3^{d} ± 0.1	14.2^{bcd} ± 0.3	13.5^{ab} ± 0.3	19^{e} ± 1	ns	*	ns
33	Ethyl propanoate	0.8^{f} ± 0.1	0.52^{ab} ± 0.03	0.46^{a} ± 0.03	0.56^{bc} ± 0.04	0.54^{b} ± 0.03	0.68^{ef} ± 0.11	0.5^{b} ± 0.1	0.58^{bcd} ± 0.02	0.7^{ef} ± 0.1	0.65^{de} ± 0.01	0.63^{cde} ± 0.04	**	***	ns
34	Ethyl butanoate	**1.13^{e} ± 0.04**	**1.00^{c} ± 0.02**	**1.06^{b} ± 0.03**	n.f.^{a}	n.f.^{a}	n.f.^{a}	**0.9^{b} ± 0.1**	**1.06^{d} ± 0.02**	n.f.^{a}	n.f.^{a}	n.f.^{a}	ns	***	ns
35	Ethyl hexanoate	n.f.^{a}	**4.7 ^{g} ± 0.2**	**3.9^{b} ± 0.2**	**4.5 ^{fg} ± 0.2**	**4.2^{cde} ± 0.1**	**3.9^{bc} ± 0.1**	**4.2^{e} ± 0.1**	**3.9^{bcd} ± 0.1**	**4.3^{ef} ± 0.2**	**4.2^{e} ± 0.1**	**4.19^{de} ± 0.04**	ns	ns	ns
36	Ethyl octanoate	**0.29^{a} ± 0.02**	**2.2^{c} ± 0.1**	**1.91^{b} ± 0.04**	**2.4^{d} ± 0.1**	**1.9^{b} ± 0.2**	**1.8^{b} ± 0.1**	**1.8^{b} ± 0.1**	**1.88^{b} ± 0.03**	**2.4^{d} ± 0.2**	**1.9^{b} ± 0.1**	**1.9^{b} ± 0.1**	ns	ns	ns
37	Ethyl 3-hydroxyoctanoate (μg/L)[6]	0.6^{a} ± 0.0	8.1^{e} ± 0.2	9^{e} ± 1	3.5^{b} ± 0.4	8.4^{e} ± 0.4	8.2^{e} ± 0.2	6^{d} ± 1	7.9^{e} ± 0.2	4.7^{c} ± 0.3	6.5^{d} ± 0.1	6.4^{d} ± 0.4	ns	*	ns
38	Ethyl 9-decenoate (μg/L)[7]	n.f.^{a}	7.1^{f} ± 0.3	13.9 ^{g} ± 0.4	6.6^{f} ± 0.4	5^{de} ± 1	6^{ef} ± 1	1.9^{b} ± 0.2	4.7^{de} ± 0.3	2^{b} ± 1	4.4^{d} ± 0.2	2.9^{c} ± 0.3	***	ns	*
39	Ethyl decanoate (μg/L)	105^{a} ± 3	**427^{f} ± 23**	**428^{f} ± 20**	**520 ^{g} ± 17**	**182^{c} ± 17**	**283^{e} ± 18**	**168^{c} ± 9**	**234^{d} ± 18**	**513 ^{g} ± 24**	139^{b} ± 5	**270^{e} ± 12**	**	ns	*
40	Ethyl dodecanoate (μg/L)	1.9^{a} ± 0.2	14 ^{g} ± 0.2	15 ^{g} ± 1	13 ^{fg} ± 1	3.6 ^{cd} ± 0.4	11.3^{f} ± 3	3.00^{c} ± 0.01	5.7^{e} ± 0.2	4.0^{d} ± 0.2	2.4^{b} ± 0.1	5.3^{e} ± 0.4	***	ns	ns
41	Ethyl tetradecanoate (μg/L)	2.6^{a} ± 0.2	3.5^{b} ± 0.4	3.4^{b} ± 0.2	2.5^{a} ± 0.2	2.4^{a} ± 0.1	3.5^{b} ± 0.2	3.0^{a} ± 0.2	4.3^{c} ± 0.2	2.6^{a} ± 0.4	2.5^{a} ± 0.2	2.8^{a} ± 0.1	ns	**	ns
42	Ethyl hexadecanoate (μg/L)	13^{d} ± 2	28^{e} ± 0.2	25^{e} ± 2	7^{c} ± 2	n.f.^{a}	n.f.^{a}	25^{e} ± 1	47^{f} ± 1	6^{b} ± 1	n.f.^{a}	n.f.^{a}	ns	***	ns
	Σ *EEBAs* (μg/L)	445 ^{h} ± 15	465 ^{h} ± 6	30^{a} ± 2	37^{c} ± 1	74^{f} ± 3	47^{e} ± 1	419 ^{g} ± 21	32^{b} ± 1	45^{e} ± 1	41^{d} ± 2	45^{e} ± 1	ns	**	ns
43	Ethyl isobutanoate (μg/L)	7.8^{def} ± 0.1	6^{bc} ± 1	8^{def} ± 1	7^{cde} ± 1	6^{ab} ± 1	8.8^{f} ± 0.3	5^{a} ± 1	6.8 ^{cd} ± 0.1	8^{ef} ± 1	6.4^{bc} ± 0.4	8.9^{f} ± 0.1	ns	ns	ns
44	Ethyl 3-methylbutanoate (μg/L)	**430^{gh} ± 15**	**454 ^{h} ± 6**	**18^{a} ± 1**	**26^{c} ± 2**	**63^{f} ± 2**	**34^{e} ± 1**	**409 ^{g} ± 21**	**21^{b} ± 1**	**33^{de} ± 1**	**30^{d} ± 2**	**32^{de} ± 1**	ns	*	ns
45	Ethyl 2-methyloctanoate (μg/L)	7.0 ^{g} ± 0.1	5^{f} ± 0	4.68^{ef} ± 0.02	4.0^{a} ± 0.3	4.64^{def} ± 0.03	4.6^{def} ± 0.1	4.7^{ef} ± 0.1	4.8^{ef} ± 0.1	4.4^{b} ± 0.1	4.4^{bc} ± 0.2	4.5^{bcd} ± 0.2	ns	**	ns
	Σ M*EFAs* (μg/L)	127^{f} ± 14	69^{d} ± 2	40^{ab} ± 2	35^{a} ± 3	97^{e} ± 9	163 ^{g} ± 1	42^{bc} ± 5	76^{d} ± 2	44^{bc} ± 5	47^{c} ± 2	151 ^{g} ± 8	ns	ns	ns
46	Methyl acetate (μg/L)	127^{f} ± 14	63^{d} ± 2	33^{b} ± 2	28^{a} ± 3	90^{e} ± 9	155.4 ^{h} ± 0.8	37^{b} ± 4	69^{d} ± 3	34^{b} ± 4	43^{c} ± 2	143.2 ^{fg} ± 8	ns	ns	ns
47	Methyl 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl propionate) (μg/L)[8]	0.7^{a} ± 0.0	5.8^{d} ± 0.3	6.9^{e} ± 0.1	7^{e} ± 1	6.9^{e} ± 0.1	7.1^{e} ± 0.2	5^{c} ± 1	7.0^{e} ± 0.1	10^{f} ± 1	3.4^{b} ± 0.3	7^{e} ± 1	ns	ns	ns
	Σ HAAs (μg/L)	357 ^{g} ± 10	252^{f} ± 2	116^{d} ± 9	n.f.^{a}	59^{c} ± 3	n.f.^{a}	184^{e} ± 9	2.3^{b} ± 0.2	2^{b} ± 1	59^{c} ± 4	n.f.^{a}	ns	***	ns
48	Isobutyl acetate (μg/L)	n.f.^{a}	n.f.^{a}	4.1^{d} ± 0.1	n.f.^{a}	n.f.^{a}	n.f.^{a}	n.f.^{a}	2.3^{bc} ± 0.2	2^{b} ± 1	2.7^{c} ± 0.1	n.f.^{a}	ns	ns	*
49	Hexyl acetate (μg/L)	n.f.^{a}	n.f.^{a}	n.f.^{a}	n.f.^{a}	n.f.^{a}	n.f.^{a}	n.f.^{a}	n.f.^{a}	n.f.^{a}	21^{b} ± 1	n.f.^{a}	ns	ns	ns
50	2-Phenylethyl acetate (μg/L)	**357^{e} ± 10**	**252^{d} ± 2**	112^{b} ± 9	n.f.^{a}	n.f.^{a}	n.f.^{a}	184^{c} ± 9	n.f.^{a}	n.f.^{a}	n.f.^{a}	n.f.^{a}	ns	***	ns
51	Isobornyl acetate (μg/L)[9]	n.f.^{a}	n.f.^{a}	n.f.^{a}	n.f.^{a}	59^{c} ± 3	n.f.^{a}	n.f.^{a}	n.f.^{a}	n.f.^{a}	35^{b} ± 3	n.f.^{a}	ns	*	ns
	Σ IEFAs	2.23 ^{h} ± 0.03	1.7 ^{g} ± 0.2	1.17^{f} ± 0.02	1.14^{f} ± 0.12	0.013^{b} ± 0.001	0.015^{c} ± 0.001	1.2^{f} ± 0.1	0.87^{d} ± 0.02	1.0^{e} ± 0.1	0.01^{a} ± 0.00	0.015^{bc} ± 0.001	ns	***	ns
52	3-Methylbutyl acetate (isoamyl acetate)	**2.22^{f} ± 0.03**	**1.7^{e} ± 0.2**	**1.15^{d} ± 0.02**	**1.1^{d} ± 0.1**	n.f.^{a}	n.f.^{a}	**1.2^{d} ± 0.1**	**0.85^{b} ± 0.02**	**0.9^{c} ± 0.1**	n.f.^{a}	n.f.^{a}	ns	***	ns
53	Octanoic acid, 3-methylbutyl ester (isoamyl octanoate) (μg/L)[10]	9^{a} ± 0	14.4^{de} ± 0.4	17^{f} ± 1	16.3^{f} ± 0.3	13^{c} ± 1	15^{e} ± 1	11^{b} ± 1	13.9 ^{cd} ± 0.4	13^{c} ± 1	9^{a} ± 0	15^{de} ± 1	**	ns	ns
	Σ Miscellaneous (μg/L)	1^{a} ± 0	1.30^{b} ± 0.04	1^{a} ± 0	1^{a} ± 0	1^{a} ± 0	1^{a} ± 0	1.29^{b} ± 0.04	1^{a} ± 0	1^{a} ± 0	1^{a} ± 0	1^{a} ± 0	ns	***	ns
54	Hexanoic acid, 2-phenylethyl ester (μg/L)[11]	1^{a} ± 0	1.30^{b} ± 0.04	1^{a} ± 0	1^{a} ± 0	1^{a} ± 0	1^{a} ± 0	1.29^{b} ± 0.04	1^{a} ± 0	1^{a} ± 0	1^{a} ± 0	1^{a} ± 0	ns	***	ns
	Total esters	77 ^{cd} ± 4	81^{de} ± 1	77 ^{cd} ± 1	84^{e} ± 2	72^{ab} ± 2	75^{bc} ± 1	83^{e} ± 3	78 ^{cd} ± 3	78^{cde} ± 1	76 ^{cd} ± 1	70^{a} ± 5	ns	*	ns
	Σ *Lactones*	103^{f} ± 14	57^{de} ± 1	34^{a} ± 1	40^{b} ± 3	96^{f} ± 3	55^{d} ± 3	40^{b} ± 7	56^{d} ± 4	47^{c} ± 4	44^{bc} ± 3	65^{e} ± 2	ns	ns	ns
55	2(5*H*)-Furanone (γ-crotonolactone)	**103^{f} ± 14**	**57^{de} ± 1**	**34^{a} ± 1**	**35^{ab} ± 3**	**57^{de} ± 5**	**55.1^{d} ± 3**	**40^{bc} ± 7**	**56^{de} ± 4**	**40^{bc} ± 3**	**43^{c} ± 3**	**65^{e} ± 2**	ns	ns	ns

(*continued on next page*)

Table 2 (*continued*)

N°	Compound	BW	*P29* 3 M	*P29* 6 M	*P29* 9 M	*P29* 12 M	*P29* 15 M	*G1* 3 M	*G1* 6 M	*G1* 9 M	*G1* 12 M	*G1* 15 M	***Y*** *p*	***t*** *p*	***Yxt*** *p*
56	2(3*H*)-Dihydrofuranone (γ-butyrolactone)	n.f.^{a}	n.f.^{a}	n.f.^{a}	**5.5^{b} ± 0.2**	**38^{d} ± 4**	n.f.^{a}	n.f.^{a}	n.f.^{a}	**7^{c} ± 1**	n.f.^{a}	n.f.^{a}	ns	*	ns
57	2-Oxepanone (caprolactone) (μg/L)	9^{c} ± 1	n.f.^{a}	n.f.^{a}	n.f.^{a}	n.f.^{a}	n.f.^{a}	2^{b} ± 0	1.65^{b} ± 0.01	n.f.^{a}	n.f.^{a}	n.f.^{a}	***	***	***
58	γ-Nonalactone (μg/L)	**43^{h} ± 4**	21^{g} ± 2	16.3^{f} ± 0.1	7.7^{d} ± 0.1	4.03^{a} ± 0.03	6.4^{c} ± 0.3	16^{f} ± 1	15.5^{f} ± 0.3	11.5^{e} ± 0.1	4.7^{b} ± 0.4	7.2^{d} ± 0.4	ns	***	ns
59	γ-Decalactone (μg/L)	5^{e} ± 0	4.2^{ab} ± 0.1	4.4^{bcd} ± 0.1	4.1^{a} ± 0.1	4.4^{abc} ± 0.1	4.7^{e} ± 0.1	4.18^{abc} ± 0.03	5^{cde} ± 1	4.2^{ab} ± 0.1	4.3^{abc} ± 0.1	4.8^{de} ± 0.2	ns	ns	ns
	Σ Terpenoids (μg/L)	514^{g} ± 17	467^{f} ± 4	401^{ab} ± 5	439^{de} ± 16	420^{bcd} ± 21	434^{cde} ± 13	424^{cd} ± 10	387^{a} ± 14	389^{a} ± 4	415^{bc} ± 4	458^{ef} ± 27	ns	ns	ns
60	*o*-Cymene (μg/L)[12]	177^{f} ± 6	6^{d} ± 1	6^{d} ± 1	5^{c} ± 1	6.3^{d} ± 0.2	4^{b} ± 1	n.f.^{a}	n.f.^{a}	3.6^{b} ± 0.3	4^{b} ± 1	n.f.^{a}	***	ns	***
61	Limonene (μg/L)	**33^{d} ± 1**	**41^{e} ± 1**	**11^{a} ± 1**	**73^{f} ± 2**	**20.2^{bc} ± 0.1**	**24^{c} ± 1**	**46^{e} ± 3**	**12^{a} ± 2**	**19^{b} ± 1**	**21^{bc} ± 1**	**30^{d} ± 7**	ns	ns	ns
62	*trans*-Citral (μg/L)	n.f.^{a}	n.f.^{a}	n.f.^{a}	1.68^{d} ± 0.03	n.f.^{a}	1.56^{c} ± 0.02	n.f.^{a}	1.2^{b} ± 0.2	1.75^{d} ± 0.03	n.f.	n.f.^{a}	ns	ns	ns
63	Linalool (μg/L)	n.f.^{a}	**55^{f} ± 2**	**48^{e} ± 4**	**30^{b} ± 3**	**51^{ef} ± 3**	**37^{c} ± 1**	**54.3^{f} ± 0.3**	**48^{e} ± 2**	n.f.^{a}	**37^{d} ± 1**	**33^{c} ± 1**	ns	***	ns
64	α-Terpinolene (μg/L)[13]	55^{g} ± 4	55^{g} ± 2	49^{ef} ± 1	30^{b} ± 3	n.f.^{a}	37^{c} ± 1	54.0^{fg} ± 0.3	48^{e} ± 2	37^{d} ± 4	37^{d} ± 1	33^{c} ± 1	ns	***	*
65	Neroloxide (μg/L)[14]	n.f.^{a}	n.f.^{a}	n.f.^{a}	87^{bc} ± 6	103^{e} ± 11	85^{de} ± 4	n.f.^{a}	n.f.^{a}	89^{cd} ± 3	85^{bc} ± 4	80^{b} ± 2	ns	***	*
66	α- Terpineol (μg/L)	239^{cd} ± 10	293^{f} ± 8	271^{e} ± 4	201^{a} ± 6	219^{b} ± 9	209^{bc} ± 8	255^{de} ± 9	262^{e} ± 11	224^{bc} ± 3	209^{ab} ± 8	269^{e} ± 31	ns	***	*
67	*trans*-Nerolidol (μg/L)	10^{a} ± 0	16^{def} ± 0	17^{f} ± 1	13^{b} ± 1	21^{g} ± 1	21.9^{ef} ± 0.4	15^{cde} ± 1	16.6^{f} ± 0.1	15^{cd} ± 1	21.9^{g} ± 0.4	14^{c} ± 1	ns	ns	ns
	Σ Norisoprenoids	1.11^{c} ± 0.01	1.6^{f} ± 0.1	1.7^{f} ± 0.1	0.92^{a} ± 0.03	1.41^{e} ± 0.03	1.1^{bc} ± 0.1	1.2^{d} ± 0.1	1.37^{e} ± 0.04	1.01^{b} ± 0.04	0.94^{a} ± 0.1	1.09^{c} ± 0.04	*	***	ns
68	Vitispirane (μg/L)[15]	312^{a} ± 15	728^{c} ± 45	602^{b} ± 39	558.3^{b} ± 29	857^{d} ± 24	699.3^{c} ± 72	576^{b} ± 44	690^{c} ± 17	595^{b} ± 28	600.2^{a} ± 26	676^{c} ± 32	ns	ns	ns
69	β-Damascenone (μg/L)[16]	783^{e} ± 25	859^{e} ± 46	1032^{f} ± 101	346^{a} ± 15	540^{c} ± 14	325^{a} ± 47	611^{cd} ± 41	658.3^{d} ± 46	403^{b} ± 35	325^{a} ± 47	406^{b} ± 16	*	***	*
70	Geranylacetone (μg/L)[17]	14.5^{c} ± 0.3	20^{h} ± 1	18^{g} ± 1	12.6^{a} ± 0.2	15.6^{e} ± 0.2	13.5^{b} ± 0.1	17.28^{f} ± 0.04	19.7^{h} ± 0.3	14.8^{cd} ± 0.1	13.5^{b} ± 0.1	15.2^{de} ± 0.3	ns	***	ns
	Σ Volatile phenols	5.8^{d} ± 0.2	3.6^{a} ± 0.1	7.9^{e} ± 0.1	4.1^{b} ± 0.5	4.1^{b} ± 0.1	4.2^{b} ± 0.1	4.3^{b} ± 0.3	8.6^{e} ± 0.1	6.1^{d} ± 0.3	4.4^{bc} ± 0.3	4.9^{c} ± 0.2	ns	*	ns
71	Phenol (μg/L)[18]	n.f.^{a}	262^{c} ± 14	667^{g} ± 40	348^{d} ± 12	362^{d} ± 15	543^{f} ± 55	159^{b} ± 11	540^{f} ± 51	403^{e} ± 14	360^{d} ± 26	444^{e} ± 27	ns	ns	ns
72	*m*-Cresol[19]	n.f.^{a}	n.f.^{a}	0.48^{c} ± 0.03	n.f.^{a}	n.f.^{a}	n.f.^{a}	n.f.^{a}	0.37^{b} ± 0.02	n.f.^{a}	n.f.^{a}	n.f.^{a}	ns	***	ns
73	2-Methoxyphenol (guaiacol) (μg/L)	**273^{e} ± 63**	**185^{d} ± 3**	n.f.^{a}	**9^{b} ± 2**	n.f.^{a}	n.f.^{a}	n.f.^{a}	n.f.^{a}	**35^{c} ± 3**	n.f.^{a}	n.f^{a}	ns	ns	*
74	Anethole (μg/L)	n.f^{a}	271^{e} ± 4	181^{d} ± 3	166^{c} ± 2	161^{c} ± 4	n.f.^{a}	83^{b} ± 1	85^{b} ± 1	82^{b} ± 14	n.f^{a}	n.f.^{a}	***	***	ns
75	4-Ethyl-2-methoxyphenol	**0.5^{e} ± 0.1**	**0.27^{d} ± 0.03**	n.f.^{a}	n.f.^{a}	0.009^{b} ± 0.001	n.f.^{a}	**0.13^{c} ± 0.01**	n.f.^{a}	n.f.^{a}	n.f.^{a}	n.f.^{a}	ns	***	ns
76	1,3-Dimethoxy-2-hydroxybenzene	0.2^{b} ± 0.1	n.f.^{a}	n.f.^{a}	n.f.^{a}	n.f.^{a}	n.f.^{a}	n.f.^{a}	n.f.^{a}	n.f.^{a}	n.f.^{a}	n.f.^{a}	ns	ns	ns
77	2,4-bis(1,1-Dimethylethyl)phenol[20]	4.9^{d} ± 0.2	2.6^{a} ± 0.1	6.6^{f} ± 0.1	4^{b} ± 1	3.6^{b} ± 0.1	3.7^{bc} ± 0.1	3.9^{bc} ± 0.3	7.6^{g} ± 0.1	5.6^{e} ± 0.3	4.0^{c} ± 0.3	4.5^{d} ± 0.2	*	ns	ns
	Σ Furanic compounds	2.9^{cd} ± 0.2	5.5^{g} ± 0.4	1.9^{a} ± 0.1	2.3^{b} ± 0.1	2.7^{c} ± 0.2	3.0^{cde} ± 0.2	3.2^{de} ± 0.2	3.7^{f} ± 0.2	2.3^{b} ± 0.1	2.29^{b} ± 0.04	3.3^{e} ± 0.1	ns	*	ns
78	Furfuryl alcohol	2.1^{fg} ± 0.1	2.2^{g} ± 0.4	0.81^{a} ± 0.01	0.84^{a} ± 0.02	1.9^{ef} ± 0.2	1.5^{de} ± 0.1	1.2^{b} ± 0.1	1.5^{cd} ± 0.1	0.8^{a} ± 0.1	1.5^{c} ± 0.1	1.7^{cd} ± 0.1	ns	ns	ns
79	2-Furaldehyde	n.f.^{a}	**1.9^{e} ± 0.1**	**0.9^{b} ± 0.1**	**1.2^{d} ± 0.1**	n.f.^{a}	n.f.^{a}	**1.1^{c} ± 0.1**	**1.0^{c} ± 0.1**	**1.2^{d} ± 0.1**	n.f.^{a}	n.f.^{a}	ns	***	ns
80	2-Acetylfuran[21]	0.9^{f} ± 0.1	0.47^{d} ± 0.04	0.30^{c} ± 0.02	0.23^{b} ± 0.03	n.f.^{a}	0.6^{e} ± 0.1	0.26^{b} ± 0.04	0.45^{d} ± 0.04	0.33^{c} ± 0.02	n.f.^{a}	0.77^{f} ± 0.04	ns	ns	ns
81	5-(Hydroxymethyl)-2-furaldehyde	n.f.^{a}	0.86^{ef} ± 0.05	n.f.^{a}	n.f.^{a}	0.9^{f} ± 0.1	0.8^{def} ± 0.1	0.67^{b} ± 0.02	0.73^{bc} ± 0.04	n.f.^{a}	0.79^{cde} ± 0.09	0.76^{cd} ± 0.03	ns	ns	ns

(*continued on next page*)

Table 2 (*continued*)

N°	Compound	P29						G1					Y	t	Yxt
		BW	3 M	6 M	9 M	12 M	15 M	3 M	6 M	9 M	12 M	15 M	p	p	p
82	Ethyl furoate (μg/L)	$24^{b} \pm 1$	$44^{e} \pm 3$	$22^{b} \pm 2$	$25^{b} \pm 2$	n.f.[a]	n.f.[a]	$37^{d} \pm 5$	$23.5^{b} \pm 0.3$	$30^{c} \pm 4$	n.f.[a]	n.f.[a]	ns	***	ns
	Σ Polyols	$440^{ab} \pm 7$	$478^{cd} \pm 12$	$488^{d} \pm 5$	$485^{d} \pm 7$	$448^{b} \pm 11$	$542^{e} \pm 17$	$453^{bc} \pm 5$	$450^{b} \pm 4$	$525^{e} \pm 3$	$418^{a} \pm 10$	$482^{d} \pm 46$	ns	ns	ns
11	2.3-Butanediol (laevo)	$327^{a} \pm 6$	$357^{bcd} \pm 9$	$399^{e} \pm 4$	$395^{e} \pm 8$	$366^{cd} \pm 11$	$406^{ef} \pm 17$	$336^{ab} \pm 2$	$368^{d} \pm 3$	$430^{f} \pm 4$	$347^{bc} \pm 8$	$356^{bcd} \pm 34$	ns	ns	ns
12	2.3-Butanediol (*meso*)	$114^{e} \pm 1$	$121^{ef} \pm 3$	$89^{c} \pm 3$	$90^{cd} \pm 2$	$82^{b} \pm 1$	$136^{g} \pm 1$	$116^{e} \pm 4$	$82^{b} \pm 2$	$96^{d} \pm 7$	$71^{a} \pm 2$	$126^{f} \pm 12$	ns	ns	ns

a, b, c, e 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; n.f.= not found; ns= non-significant. *, **, *** indicate significance at 0.05, 0.01 and 0.001 level, respectively. Identification of wine samples: BW: base wine. 3M: sparkling wines with 3 months of ageing. 6M: sparkling wines with 6 months of aging. 9M: sparkling wines with 9months of ageing. 12M: sparkling wines with 12 months of aging. 15M: sparkling wines with 15 months of ageing. P29: *S. cerevisiae P29 strain*. G1: *S. cerevisiae G1 strain*. † Isoamyl alcohols = 2-methylbutanol + 3-methylbutanol. [1]Quantified as furfural. [2]Quantified as octanal. [3]Quantified as caprolactone. [4]Quantified as benzaldehyde. [5]Quantified as hexadecanoic acid. [6]Quantified as ethyl octanoate. [7]Quantified as ethyl decanoate. [8]Quantified as ethyl hexadecanoate. [9]Quantified as 2-phenylethyl acetate. [10]Quantified as ethyl undecanoate. [11]Quantified as ethyl dodecanoate. [12, 13]Quantified as limonene. [14]Quantified as terpineol. [15,16]Quantified based on internal standard. [17]Quantified as geraniol. [18,19]Quantified as guaiacol. [20]Quantified as 4-ethyl-2-methoxyphenol. [21]Quantified as 5-methylfurfural. EEFAs: Ethyl esters of fatty acids: EEBAs: Ethyl esters of brancched acids, MEFAs: methyl esters of fatty acids; HAAs: higher alcohol acetates: IEFAs: isoamyl esters of fatty acids. *Y*: yeast factor. *t*: time factor. *Yxt*: interaction. **In bold, the concentrations of compounds having odour activity value (OAV) >1.**

(Table 2) and mostly contribute to fruity, balsamic, vegetable, empyreumatic and spicy series. Several studies comparing BW with their corresponding aged sparkling wines, describe the aroma of the former as fruity, with tropical and citric nuances, as a consequence of higher amount in acetates (Torrens et al., 2008; 2010). Welke et al. (2014) also found ethyl esters, higher alcohols, carbonyl compounds and volatile phenols as mainly responsible for the differences between BW and aged sparkling wines.

Cluster 4 comprises three carbonyl compounds (**18**, **20**, **25**), an ester (**44**), a monoterpene (**61**) and a furfuryl compound (**78**), while cluster 6 (with 10 compounds) brings together terpenoids and norisoprenoids. From them, six compounds showed OAV > 1 (**20**, **21**, **34**, **44**, **61** and **69**) that display the highest contents in 3 M wines, predominantly in P29 yeast, contributing to the fruity and floral aroma. These series are also included for the ten compounds in cluster 7, that are present at the highest content in 12 M aged wines. In contrast, cluster 5, which reaches its peak in 15 M−aged wines, contributes compounds **19**, **24**, **46**, **55**, **59**, **80** to the same series in addition to empyreumatic and fatty series. This agrees with the results of other authors who stated that floral, fruity, sweet, toasty, lactic and yeasty nuances become more complex with ageing, as a consequence of the increase in esters, alcohols and some varietal compounds as well as a decrease in acetates and fatty acids (Torrens et al., 2010; Pérez-Magariño et al., 2013). Lastly, cluster 8, with nine compounds (most of them contributors to fatty series) shows high quantity in 6 M sparkling wines, most of them being characterised by fatty/metallic nuances.

3.4. Sensory analysis

This section covers the results for the triangle test (*t*-test) and the descriptive test 1 (DA1-test), while DA2-test will be explained in a later section. The *t*-test was used in our study to determine sensory differences or similarities between samples obtained by each yeast tested at the same ageing time and the number of correct *versus* the total number of judgements were subjected to statistical analysis (Table 3). According to these results, panellists detected significant differences in the five-wine series tested and the higher number of correct answers were obtained when comparing the wines aged 3, 6 and 9 months. This suggests a trend towards the homogenisation of sensory attributes in sparkling wines when they exceed 12 months of ageing, regardless of the yeast used, and it could be related to the aforementioned observations for colour parameters and volatile fractions.

Fig. 1 shows the results for DA1-test (OIV, 2009), through the dataset previously scaled, in a radar plot for the twelve attributes subjected to evaluation for each yeast tested. The ANOVA shows significant differences related to ageing for aroma intensity (A.I.) in P29 wines and, limpidity (L), taste intensity (T.I.), persistence (P) and characteristic taste (C.T.) for G1 wines. Although ageing had a strong influence on the colour, no differences were found because the visual term used in DA1-test also entails foaming characteristics. In contrast, most of the

Table3
Sensory triangle tests performed by 12 tasters in sparkling wines with different ageing time.

Months of ageing on yeast lees	Compared wines	Correct answers (*n*)[a]	Percentage of correct answers
3	P293M-G13M	8***	66.7
6	P296M-G16M	9***	75.0
9	P299M-G19M	9***	75.0
12	P2912M-G112M	7**	58.3
15	P2915M-G115M	6*	50.0

*, **, *** indicate significance at 0.05, 0.01 and 0.001 level, respectively. The statistical results were obtained by applying chi-squared test and the maximum number of correct answers expected '*n*' was 12.

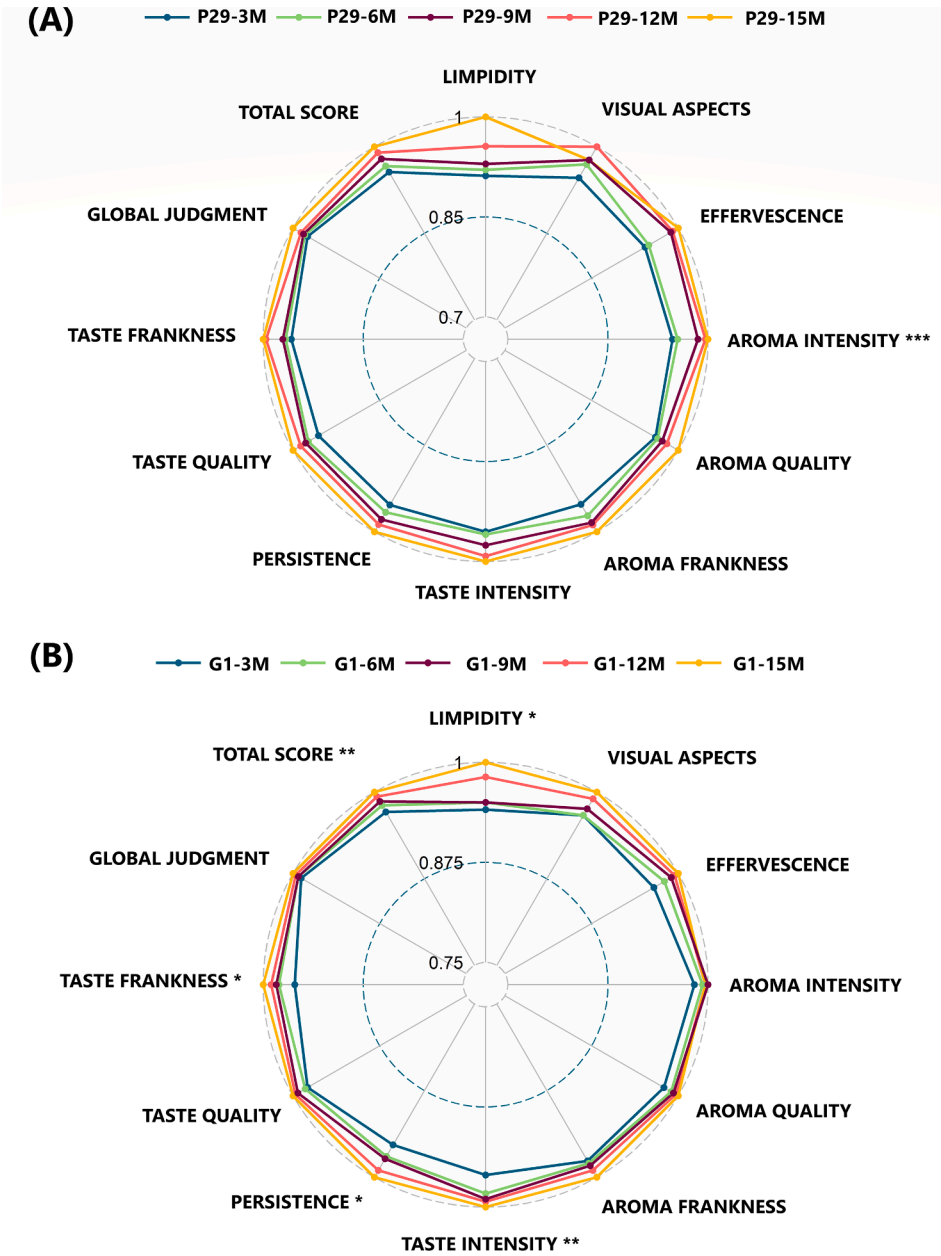

Fig. 1. Sensory analysis of sparkling wines made with (A) P29 yeast strain or (B) G1 yeast strain and obtained by the mean of the scores given by the panellists for each descriptor (data were previously scaled). Cyan lines: 3 months (3 M). Green lines: 6 M months (6 M). Magenta lines: 9 months (9 M). Light Red lines: 12 months (12 M). Yellow lines: 15 months (15 M). (*, **, ***) Significant differences among sparkling wine samples at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

attributes show differences ($p \leq 0.05$) when the two yeasts are compared (results not shown); exceptions are effervescence (E), aroma quality (A. Q.), characteristic aroma (C.A.) and taste quality (T.Q.). The results found here for visual aspect and effervescence, are in line with those obtained by Ubeda et al. (2019), since no differences in foamability, crown formation, persistence, bubble speed and size were established for sparkling wine with the same ageing period. The differences observed for T.I. and P could be related to changes in the protein content of wines (González-Jiménez et al., 2020). As a general rule, wines made using each yeast obtain higher scores and become more similar as ageing increases; as seen in *t*-test. This fact may be related to the highest contents in higher alcohols and volatile phenols in young wines (Torrens et al., 2008). Furthermore, it is important to highlight that P29 wines displayed a lower overall score (70–82 points) and also lower limpidity, compared to its G1 counterparts (77–87 points). Changes in limpidity are justified by the high capacity to form flocs of G1 yeasts (Martínez-García et al., 2020), since no fining agent were used during 'tirage' to avoid changes in the aroma, as stated by García et al. (2009) and Puig-Pujol et al. (2013). The total score obtained allows classification of all the wines from good to very good in terms of overall quality.

3.5. Volatilome dataset vs. Sensory profile

Two approaches were carried out through the use of unsupervised and supervised pattern recognition techniques. The first one establishes differences between the wines, diminishing redundancy and dimensionality; the second technique helps to predict the behaviour of each sensory attribute, based on the most contributing compounds.

3.5.1. Principal component analysis (PCA) for odorant series and sensory analysis data

Different PCA models were built through two datasets: the values of the eight-odorant series (OS), obtained from each quantified compound, and the results of the aroma descriptive test 2 (DA2) that comprise the

same sensory attributes (Supplementary Table 2). For this, those compounds without specific calibration curve or perception threshold were excluded from this analysis (**22**, **23**, **24**, **25**, **32**, **37**, **38**, **45**, **47**, **51**, **53**, **54**, **60**, **64**, **65**, **68**, **69**, **70**, **71**, **72**, **74**, **77** and **80**). After the feature extraction, pre-processing was performed on the data (mean centring and scaling). To begin with, the correlation between odorant series (OS) and sensory attributes (SA) was studied as a tentative approach. Thus, the obtained correlation coefficients (*r*) between OS-SA were 0.91, 0.90, 0.89, 0.87, 0.85, 0.85, 0.75 and 0.70 ($p < 0.001$), for floral, chemical, spicy, fruity, fatty, vegetal, balsamic and empyreumatic, respectively.

Fig. 2 shows the PCA obtained for both data matrices. Regarding the OS model, three components were found to be relevant and explained 89% total variance; meanwhile the first two components explain about 81%. The samples scores and the loadings plot for PC1 and PC2 are shown in Fig. 2A.1 and Fig. 2A.2, respectively. It can be noticed a clear separation in this dataset, allowing an easy differentiation among the samples in relation to ageing and, to a lesser extent, yeast strain (Fig. 2A.1). Thus, the youngest sparkling wine samples (P29-3 M and G1-3 M) are located in the first quadrant of the plane generated by both components, while the remaining wines are located in the second (12 M and 15 M) and third quadrant (6 M and 9 M). Yeast effect is less clear along PC1, mainly in the oldest sparkling wines, although G1 wines always display lower scores for this axis. A similar trend is observed for this factor along PC2; an exception is made for G1-3 M sparkling wines that are located on the top of this component. The differences in wine distribution with yeast factor may be explained based on the compounds with OAV > 1 (bold letters in Table 2). López de Lerma et al. (2018) used the same strategy to differentiate the cava wines obtained with two yeast strains applied in various inoculation formats, obtaining 80.2% of the explained variance for the first two components. In the current study, compounds with the greatest odorant contribution that allow differentiating yeast effect in the 3-month ageing wines are **6**, **14**, **21**, **25**, **58**, **73**, **78**, and **79**. Similarly, compounds **8**, **28**, **35,** and **38** can be attributed at 9 months, and compounds **15**, **44**, **55**, and **56** at 12 and 15-month of ageing.

Fig. 2**A.2** shows coloured in "blue" the variables of less importance for the first two PCs and, in "red", those with a high level of representation. Thus, PC1 correlates positively with all the OS, mainly with fruity (+0.95), empyreumatic (+0.90), vegetal (+0.87), balsamic (+0.87) and spicy (+0.87). In contrast, PC2 correlates with the OS: floral (+0.67), spicy (+0.36), and fatty (–0.56). A similar distribution was obtained by Ubeda et al. (2019) for País grape sparkling wines (0 to 12 months of ageing) through a PCA model (60.7% cumulative variance) built with 57 volatile compounds, where a decrease in esters and varietal compounds (main contributors of the floral and fruity series) was observed with ageing.

With regard to PCA of DA2-dataset, three components were found to be relevant and explained>84% total variance. PC1 explained 57% variance and the sensory attributes (SA): SA-chemical (+0.86), SA-Fruity (+0.86), SA-Balsamic (+0.81) and SA-Vegetal (+0.76) had the strongest effect on it (Fig. 2B.2). In addition, PC2 accounted for 19% variance, sensory attributes SA-Floral (+0.74), SA-Balsamic (–0.43), SA-Empyreumatic (–0.52) and SA-Fatty (–0.52) being the greatest contributors. The SA scores plots (Fig. 2B.1) for the wine samples shows similar allocation to those obtained with OS-dataset, although there are slight differences. In this way, PC1 groups the wines in relation to ageing, placing youngest wines (3 M and 6 M) between the first and second quadrants. Like the OS-model, 9 M sparkling wines are located in

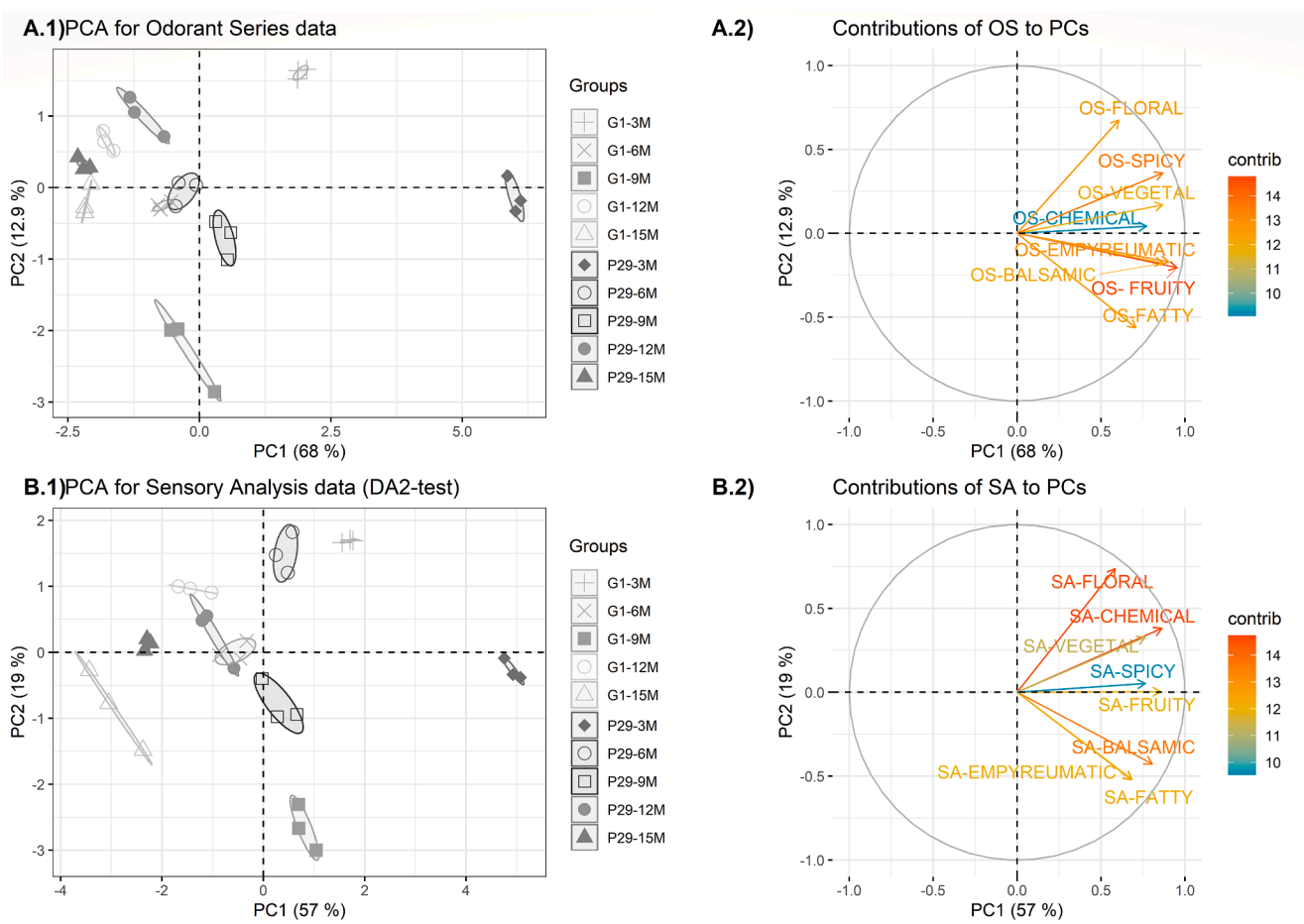

Fig. 2. Principal component analysis performed with: (A) Odorant series (OS) and (B) Sensory analysis (SA) datasets. Figures A.1 and B.1 shows the scores plot and figures A.2 and B.2 shows the loadings plots for OS and SA data, respectively. For identification of wine samples see Table 2.

the negative part of PC2. In contrast, in DA2-model the placement of 6 M wines changes slightly compared to their counterparts in the OS-PCA model. Differences due to yeast strain can be appreciated for all the wines in both axes, since scores distribution for P29 wines is more positive than G1 samples, exception made for G1-3 M and G1-12 M wines that showed the opposite trend through PC2.

According to the findings, the PCA obtained by both datasets allowed a good separation of the wine samples with respect to ageing and yeast strain factor, although DA2-model showed a higher separation power than the OS model against the yeast effect. Furthermore, both models show that P29-3 M wines are fruity, while the most floral wines are also vegetable (G1-3 M, P29-6 M) and the fattiest, both empyreumatic and balsamic (P29-9 M, G1-9 M). The lack of a clear contribution in the spicy and chemical series is justified because not all the identified compounds could be quantified and then included in the OS model, which also cannot report information on their interactions.

3.5.2. Predictive PLS models of sensory analysis dataset

Since odorant series could not be calculated with all the compounds identified, separate PLS models were built, as a tentative approach, to estimate the 8 aroma attributes (indicators) from the DA2-test. In this regard, Herrero et al. (2016) suggest a selection criterion of volatile compounds (OAVmax $\geq$ 1 and OAVmax/OAVmin $\geq$ 1.5) that is based on their ability to induce differences in sensory perception. However, the application of a less strict criterion makes it possible to avoid the loss of information provided by instrumental analysis. Thus, an input data matrix was built from the entire group of samples and the volatiles (with or without calibration) appointed to each odorant series (Supplementary

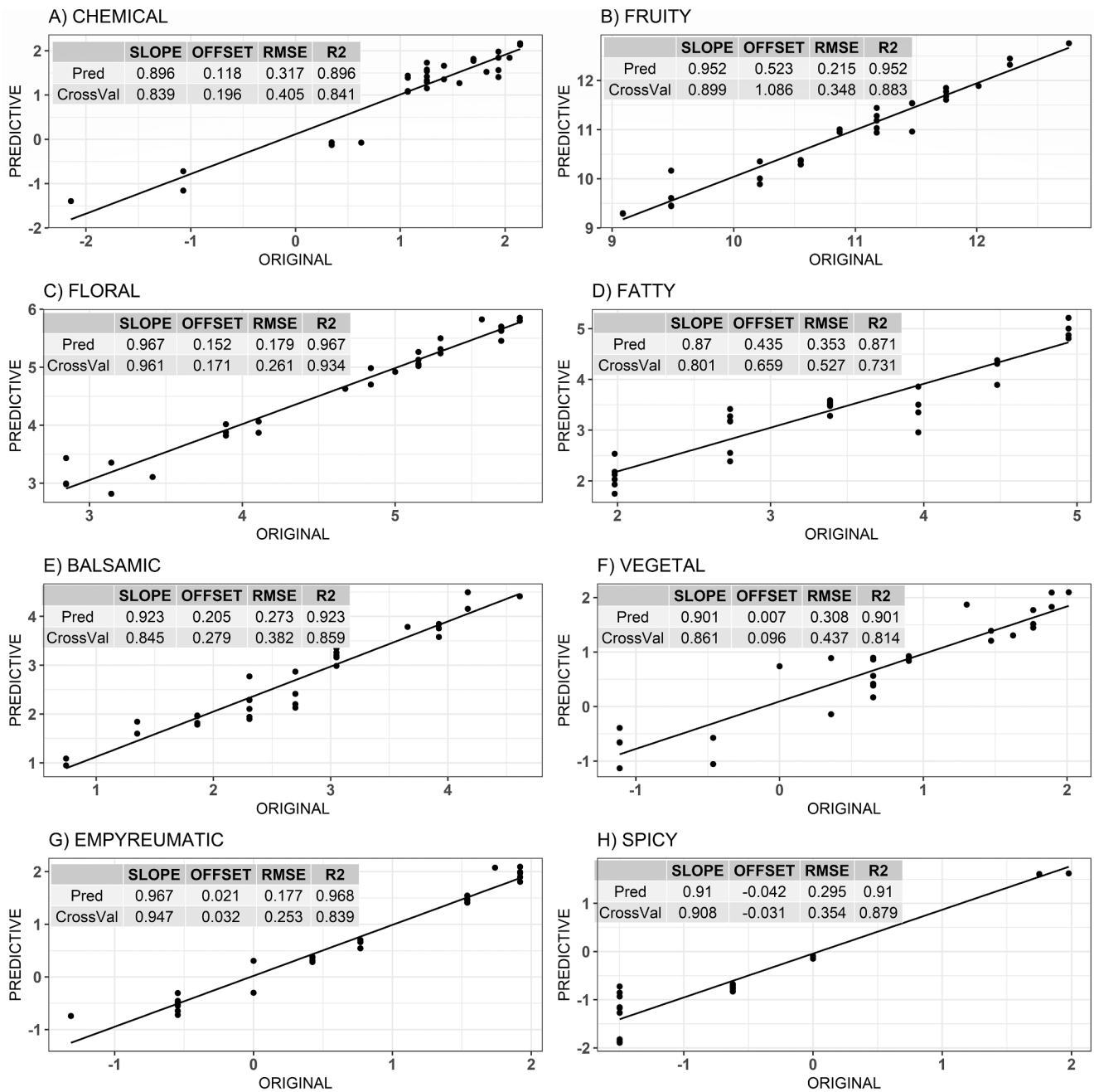

Fig. 3. Scatter plots (observed vs. predicted values) of 8 sensory attributes: (a), (b), (c), (d), (e), (f), (g) and (h) from Partial Least Square Regression (PLS) models built for all 30 sparkling wines obtained with the two yeast strains at different ageing time. Pred.: training set; Cross Val: internal validation set; R^2: goodness-of-fit in prediction for all observations in training set and internal validation set (also called Q^2). RMSE: Root Mean Squared Error.

Table 1). In this way, the model for SA-chemical attribute was built with the scores resulting from their sensorial evaluation and the contents of compounds **1**, **4**, **6**, **8**, **20**, **37**, **71**, **77**, **78**, **79**; and so on with the remaining indicators. Predictors and response variable were log_{10}-transformed and scaled, to respectively achieve a normally distributed population and give equal importance to each variable. Leave-one-out cross-validation (LOOCV) was used to determine how many latent variables (LV) are needed to find a PLS model with adequate complexity in terms of number of components, and to validate the predictive ability of resulting models. The selection of a number of LVs is focused to improve the cross-validation goodness of prediction (Q^2) and percentage of the explained variance in the response variables and minimise the cross-validation root mean squared error (RMSECV).

In light of the above mentioned, for SA-chemical, SA-fruity, SA-floral, SA-fatty, SA-balsamic, SA-vegetable, SA-empyreumatic and SA-spicy predictive models, 2, 4, 5, 3, 5, 5, 4 and 2 LVs were considered as optimal, respectively. The addition of further LVs did not improve the explained variance significantly.

Fig. 3 shows scatterplots of observed and predicted sensory attributes, the root mean squared errors and the predictive power (RMSE) of the PLS regression model developed. According to the values of R^2 (goodness of fit) and Q^2 (cross-validated goodness-of- prediction), the best model was SA-floral (0.97 and 0.93, respectively), followed by SA-fruity (0.95, 0.88), SA-spicy (0.91, 0.88), SA-balsamic (0.92, 0.86), SA-empyreumatic (0.97, 0.84), SA-chemical (0.89, 0.84), SA-vegetal (0.90, 0.81) and SA-fatty (0.87 and 0.73). The RMSECV for these models (shown in Fig. 3) are slightly higher than those obtained by Herrero et al. (2016), with similar descriptors and LVs, in Chardonnay (0.06–0.33) and Pinot noir (0.10–0.38) base wines.

Among the possibilities to describe the relative importance of the predictor compounds in the response variable, the one based on exploring their VIP values and regression coefficients (b) can be considered the most complete and easiest. As a rule-of-thumb, predictors with VIP ≥ 1 are considered as the most influential in the model, while regression coefficients are used to show the direction of the relationship. Regarding this, the highest VIP value for SA-chemical was found for compounds **4** (VIP = 1.64; b = 0.21) and **3** (VIP = 1.60; b = 0.20), followed by **6** (VIP = 1.41; b = 0.18), **2** (VIP = 1.36; b = 0.17), **79** (VIP = 1.14; b = 0.14), most of them higher alcohols with positive contribution to those wines of<15 months (***Supp. Fig. 5.A***). In contrast, SA-chemical seems to be lower with higher amount of compound 1 (methanol) due to the negative regression coefficient (b = –0.13). This trend may be attributed to the pectinolytic activity of yeasts to produce methanol with ageing (Martínez-García et al., 2020). From the 31 compounds selected for SA-fruity model, only 11 showed VIP ≥ 1 (**4**, **9**, **10**, **21**, **34**, **42**, **50**, **52**, **58**, **64** and **69**), most of them esters, with isoamyl acetate (**52**) being the greatest contributor and diethyl succinate (**10**) the one with negative correlation (b = –0.06). Isoamyl acetate has been described by Ruiz-Moreno et al. (2017) as a potential marker of young sparkling wines, which explains its high contribution to this series. At the same time, Herrero et al. (2016), working with varietal base wines, also found negative correlations between acetaldehyde and tropical fruits or citric terms, β-damascenone with floral and ethyl isobutyrate with citric attributes. Concerning SA-floral, compounds **63**, **65**, **69**, **50**, **70**, **5**, **10**, and **37** (mainly terpenoids and norisoprenoids) contribute most to it, only diethyl succinate (**10**) and neroloxide (**65**) being those with negative correlations. Again, this is explained by the increase of these compounds with ageing, compound **10** being previously proposed as a potential marker of this stage (Pozo-Bayon et al., 2003). Nevertheless, Ubeda et al. (2019) identified diethyl succinate, along with β-phenylethanol, as the main compounds responsible for floral attributes in País grape sparkling wines. On the other hand, long-chain carboxylic acids (**26**, **27**, **28** and **32**), their ethyl esters (**36** and **42**) and γ-nonalactone (**58**), were the greatest positive predictors of SA-fatty, while hexyl acetate (**49**) and *trans*-nerolidol (**67**) were the most negative (*Supp. Fig. 5.D*). This is in agreement with the high involvement of these compounds in the characteristic fruity and flowery aroma of some young white wines (Torrens et al., 2010; Muñoz-Redondo et al., 2020). Regarding SA-balsamic, three volatile phenols (**73**, **74** and **75**) and a furanic compound (**82**) exceeded VIP > 1, all of them positively correlated with the P29-3 M wines. These chemical families are also involved in SA-empyreumatic model with compounds **73**, **74** and **79**, as well as compounds belonging to EEFAs (**9**, **39**), IEFAs (**52**), lactones (**56**) and the carbonyl compounds **18** and **24** (b = –0.07); however, no available information has been obtained for the latter. Finally, compounds **14** (VIP = 2.23, b = 0.29) and **61** (VIP = 1.39, b = 0.19) for SA-Vegetal, and **44** (VIP = 1.63, b = 0.76) for SA-spicy, displayed the highest VIP values for these series, all of them characteristic of the youngest wines (Table 2).

According to the criteria mentioned in *Section 2.4.3* applied to the quantified compounds (results not shown), eight compounds were considered constitutive (**4**, **5**, **6**, **8**, **34**, **35**, **36**, **52**) and nine as discriminant (**14**, **20**, **27**, **28**, **37**, **44**, **56**, **61**, **73**) of sparkling wines obtained by both yeasts. Furthermore, in P29 set more constitutive compounds (**15**, **16**, **21**, **50**, **55**, **63** and **68**) were observed than in G1 set (**79**); the number of specific compounds with discriminant capacity for P29 (**75**, **79**) and G1 (**55**, **63**) being the same. As can be seen, a compound can play a dual role, since it is constitutive for one yeast and discriminant for the other, as said by Herrero et al., (2016).

Based on the results, 38 compounds (quantified or not) were considered the most influential for PX sparkling wines aroma, while only 18 quantified compounds stand out with OAV > 1 (bold letters in Table 2). From them, twelve display discriminant power, while only six are constitutive (**4**, **5**, **6**, **34**, **36** and **50**). Additionally, eleven compounds (**4**, **9**, **10**, **42**, **50**, **52**, **58**, **69**, **73**, **74** and **79**) have a positive influence on more than one response variable, the exception being diethyl succinate. This could be explained by the additive/suppressive effect that other compounds have on them during wine tasting.

4. Conclusions

This study focuses on analysing physicochemical and sensory differences of PX-sparkling wines obtained, according to the traditional method, with two yeasts and aged for 15 months. Univariate statistical analysis shows that ageing has a more pronounced effect on the volatilome than the yeast strain used. By contrast, PCA models obtained from odorant series and aroma descriptive tests, showed good separation of the wine samples for both factors. In addition, the sensorial evaluation provided higher scores for the wines obtained with the native yeast at any ageing time.

Finally, the study establishes interesting associations between the content in volatile compounds and the odour attributes, revealing a strong influence of thirty-eight compounds in the PX-sparkling wines aroma. Further studies are needed to consolidate the key-markers exposed here to assess the quality of PX sparkling wines in the presence of CO_2.

CRediT authorship contribution statement

Rafael Martínez-García: Formal analysis, Writing - original draft. **Juan Carlos Mauricio:** Conceptualization, Writing - review & editing. **Teresa García-Martínez:** Writing - review & editing. **Rafael A. Peinado:** Conceptualization, Writing - review & editing, Methodology. **Juan Moreno:** . **:** Funding acquisition, Supervision, Conceptualization, 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

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2021.129784.

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