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Study of the changes in volatile compounds, aroma and sensory attributes during the production process of sparkling wine by traditional method

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ABSTRACT

One of the strongest factors that affects the volatile profile of sparkling wine is the winemaking process. Here we focus on determining the effects of the second fermentation and aging on lees of sparkling wine from País grape variety combining different analysis techniques for the first time in sparkling wine: gas chromatography/mass spectrometry/olfactometry and sensorial analysis. During the second fermentation and aging, there was a significant loss of esters that might be related to the adsorption on lees and ester volatility and chemical hydrolysis. The concentration of several compounds such as some esters (diethyl succinate, ethyl lactate, and ethyl isovalerate) increased during aging and could be used as aging markers. Vitispiranes were identified as the best norisoprenoids aging markers for young sparkling wines (12 months of aging). Also, PCA showed that time of aging on lees affected mostly esters and terpenes. On the other hand, the diminution of fruity/floral impact odorants during aging was not perceived in sensorial trials. Our results suggest that the responsibility for fruity/floral nuances in sparkling wine might reside in a few high-impact aromatic compounds, such as ethyl isobutyrate, isoamyl acetate, ethyl hexanoate, β-phenylethanol and diethyl succinate.

1. Introduction

Currently, the wine whose production has increased the most is the sparkling wine (OIV. The International Organization of Vine and Wine, 2017). Thus, in the last 10 years, sparkling wine production has increased above 40% instead still wine a 7%. This is partly due to the change of the trends of consumption, from mainly festive consumption to more regular consumption (OIV. The International Organization of Vine and Wine, 2017). The wine industry is one of the most representative and productive sectors of Chile, which is among the ten countries with the highest production of wine worldwide and ranked fourth as an exporting country (OIV, 2017). During the last 10 years, sparkling wine exports have increased, reaching 5.1 million liters in 2016, which is triple that of 2006 (ODEPA. Oficina de Estudios y Políticas Agrarias, 2017).

There are two principal production methods for sparkling wine: Charmat and Traditional (*Champenoise*). These methods vary depending on the production technology that is used (Torresi, Frangipane, & Anelli, 2011). Traditional production involves a second fermentation inside of the bottle, followed by a period of contact with lees. During this aging period, several compounds (including lipids, carbohydrates, nucleotides, amino acids, peptides, mannoproteins and volatile compounds) are released in the autolysis process (Alexandre & Guilloux-Benatier, 2006). These molecules influence the characteristics of the wine and its sensorial quality (Alexandre & Guilloux-Benatier, 2006; Martínez-Rodríguez, Polo, & Carrascosa, 2001). Sparkling wine quality depends on several parameters, such as foam characteristics (foamability, persistence, in mouth aggressiveness, and bubble size), color, acidity and aroma. Aroma is considered one of the most important indicators of sparkling wine quality (Campo, Ferreira, Escudero, & Cacho, 2005; Kemp, Alexandre, Robillard, & Marchal, 2015; Pérez-Magariño, Ortega-Heras, Martínez-Lapuente, Guadalupe, & Ayestarán, 2013). Aroma is a complex character, resulting from a long sequence of biological, biochemical, and technology processes, producing hundreds of compounds in concentrations ranging from ng L^{-1} to mg L^{-1} (Bayanove, Baumes, Cruzet, & Gunata, 2000; Sagratini et al., 2012). The volatile composition of sparkling wines is influenced by several factors, including grape variety, the maturity of the grape, the

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production method, first and second fermentation process, choice of yeast, and time of aging on lees (Pozo-Bayón, Martínez-Rodríguez, Pueyo, & Moreno-Arribas, 2009). Of these, the second fermentation and the period of contact with lees have been described as having a major influence on the volatile composition in sparkling wines (Francioli, Torrens, Riu-Aumatell, López-Tamames, & Buxaderas, 2003; Pozo-Bayón et al., 2009; Riu-Aumatell, Bosch-Fusté, López-Tamames, & Buxaderas, 2006). During the autolysis yeast period, several volatile compounds are released to the wine, mainly esters, as well as some enzymes, such as glucanases which interact with the glycosidically bound aroma compounds to release the volatile molecules to the wine. Moreover, some of the volatile compounds might be adsorbed on lees, decreasing their concentration in the sparkling wines during aging (Comuzzo, Tat, Tonizzo, & Battistutta, 2006; Ganss, Kirsch, Winterhalter, Fischer, & Schmarr, 2011). Therefore, the aging time determines the type and the quantity of volatile compounds present in sparkling wines (Riu-Aumatell et al., 2006).

The grape variety has an important influence on the resulting product. Those grapes most used to produce sparkling wine are Pinot Noir, Chardonnay, Pinot Meunier, Glera, Macabeo, Xarel lo, and Parellada. However, in response to the growing demand for wines with unusual characteristics, other grape varieties are being tested to produce high-quality sparkling wines. This is the case of País grape variety, which was the first grown in Chile during the mid-16th-century and originates from Tenerife, Spain. Cultivation of the País grape has been gradually decreasing since the 19th-century because of the introduction of French varieties (Lacoste et al., 2010). Today, the País grape is the second most grown red variety in Chile (12,520 ha), being mainly grown by small farmers in the rain-fed areas of the regions of Maule and Bíobío (ODEPA, 2015).

Sparkling wines made from white grape varieties have been well-studied (Bosch-Fusté et al., 2007; Francioli et al., 2003; Gallardo-Chacón, Vichi, López-Tamames, & Buxaderas, 2010; Ganss et al., 2011; Ibern-Gómez et al., 2000; Kemp et al., 2015). However, little is known about sparkling wine produced from red grape varieties, and specifically about sparkling wine from red varieties vinified as *Blanc de noirs*. To our knowledge, no studies have been carried out on the study of impact aroma compounds during the production of sparkling wine from base wine going through aging on lees. Therefore, the aim of this study was to determine the effects of the second fermentation and the aging on lees on the composition of volatile compounds and sensorial perception of sparkling wines produced using the País grape variety.

2. Material and methods

2.1. Samples

The samples were supplied by Viña Miguel Torres Chile. Sparkling wines were produced by the traditional method *Blanc de noirs* from País cv. grape must (2015 vintage) employing the *Saccharomyces cerevisiae* ex r.f.. *bayanus* yeast (Enartis, Chile) for the second fermentation. The second fermentation was completed in around 25 days and this process and the aging on lees were carried out at 14 ± 2 °C. The samples analyzed were the País cv. must (M), the base wine (BW), and 0 (0M), 3 (3M), 6 (6M), 9 (9M), and 12 months (12M) in contact with lees, after hand disgorging and addition of liqueur d'expedition to ensure a Brut category. Samples M and BW came from a stainless-steel tank. The BW was placed in bottles to carry out the second fermentation and three bottles of each aging time were analyzed. For volatile compounds analysis and olfactometry analyses, samples were stored in amber vials at −20 °C, and for sensorial trials, the samples were stored at 15 °C until evaluations.

2.2. Reagents and standards

The standard compounds employed in this study for the identification and quantification were supplied by Sigma-Aldrich (Germany): ethyl butanoate, ethyl-2-methylbutanoate, ethyl isovalerate, ethyl hexanoate, ethyl lactate, methyl octanoate, ethyl octanoate, ethyl decanoate, diethyl succinate, isoamyl octanoate, isoamyl decanoate, isoamyl acetate, hexyl acetate, β-phenylethyl acetate, isobutanol, isoamyl alcohol, hexanol, cis-3-hexenol, 2,3-butanediol, decanol, β-phenylethanol, hexanal, furfural, linalool, α-terpineol, citronellol, nerolidol, hexanoic acid, octanoic acid, decanoic acid, ethyl undecaonate and isobutyl acetate. Sodium chloride and 4-methyl-2-pentanol (internal standard), were purchased from Merck (Darmstadt, Germany).

2.3. Enological parameters

Parameters such as pH, volatile acidity (VA), reducing sugars (RS), and alcoholic degree (°A) were determined following official analytical methods of the OIV (OIV, 2014). The color intensity (CI) and %Yellow were estimated as described by Glories (1984). The CIELab coordinates were determined according to Ayala, Echávarri, and Negueruela (1997), and data were processed with MSCV® software (Ayala, Echávarri, & Negueruela, 2014).

2.4. Headspace solid phase microextraction- gas chromatography-mass spectrometry conditions (HS-SPME-GC-MS)

The volatile compounds were extracted by Headspace Solid Phase Microextraction (HS-SPME). For this purpose, 7.5 mL of sample was placed into a 20-mL glass vial with 1.5 g of sodium chloride and 10 µL of 4-methyl-2-pentanol (0.75 mgL^{-1}) (used as internal standard), which was then transferred to an autosampler tray following the method described in Ubeda, del Barrio-Galán, Peña-Neira, Medel-Marabolí and Durán-Guerrero (2017a).

Static headspace sampling was done after the fiber was cleaned and conditioned following the manufacturer instructions (1 h at 270 °C). After 20 min incubating at 45 °C and 500 rpm agitation speed, a 2 cm 50/30 µm Carboxen/DVB/PDMS SPME fiber (Supelco, Bellefonte, p.a., USA) was exposed to the headspace of the vial for 40 min. Fiber penetration into the vial was 30 mm. Once the adsorption/absorption finished, the fiber was desorbed in the injector using the splitless (3 min) mode and a transfer line temperature of 280 °C.

Next, gas chromatography analysis was carried out using a 7890B Agilent GC system coupled to a quadrupole mass spectrometer Agilent 5977 inert (Agilent Technologies, Palo Alto, CA, USA).

A DB Wax capillary column (60 m × 0.25 mm, and 0.25 µm film thickness) (J&W Scientific, Folsom, CA, USA) was used, and the carrier gas was Helium at a flow rate of 1 mL/min. The oven temperature program was as follows: 35 °C for 1 min, then increased to 130 °C at 12 °C/min and held for 1 min, then to 160 °C at 1 °C/min, and then to 220 °C at 10 °C/min (held for 10 min). The electron ionization mass spectra in the scan mode were recorded at 70 eV with the electron energy in the range of 35 to 300 amu.

All data were recorded using an MS ChemStation (Agilent Technologies, Palo Alto, CA, USA). Moreover, blank runs using empty glass tubes were performed before and after every three runs.

2.5. Gas chromatography-olfactometry conditions (GC-O)

Olfactometry analyses were performed for BW, 0M, and 12M samples and were conducted using a 7890B Agilent GC system coupled to a quadrupole mass spectrometer Agilent 5977 inert (Agilent Technologies, Palo Alto, CA, USA) and an OP275 olfactometer (GL Science Inc., Tokyo, Japan). HS-SPME was employed as the extraction technique in the same conditions used for the volatile compounds analysis.

For this purpose, it was used an HP5-MS column (30 m × 0.25 mm × 0.22 µm film thickness) (J & W Scientific, Agilent

Technologies Inc., Santa Clara, USA). The oven temperature program was as follows: 50 °C for 1 min, increasing to 70 °C at 4 °C/min and held for 1 min. Then, the temperature was increased to 150 °C at 6 °C/min, where it was held for 2 min, and finally increased to 230 °C at 40 °C/min, and held for 3 min. The carrier gas was N_2 at a flow rate of 1 mL/min. The electron ionization mass spectra in the scan mode were recorded at 70 eV with the electron energy in the range of 35 to 300 amu.

The sensory panel consisted of six tasters (Two males and four females), all of which sniffed each sample twice, assigning to each perceived odor an intensity level: 1, 2 or 3. Results were expressed as "modified frequency" (MF), calculated with the formula proposed by Dravnieks (1964).

2.6. Identification of aroma compounds

Compound identification was based on matching using the 2.0 version of the standard NIST library and the retention index (LRI) of authentic reference standards. Linear retention indices (LRIs) were calculated by retention times of n-alkanes (C6-C30) under identical conditions for each analysis program.

Data were expressed in concentration ($\mu g L^{-1}$) obtained from calibration curves with these reference standards (relative area vs. concentration). The relative area was calculated by dividing the peak area of the major ion of each compound by the peak area of the major ion of the internal standard.

2.7. Sensorial analysis

The aroma samples of sparkling wine (0M, 3M, 6M, 9M, and 12M) were evaluated by a trained panel composed of 16 tasters (seven females and nine males). Preliminary tasting sessions were performed to select by consensus the attributes to train the panel. This panel was trained in several sessions in visual (foamability, bubble rate, bubble size, color intensity, red component, foam stability), olfactory (aromatic intensity, fruity, floral, bread/toasted/yeast, complexity), and in mouth attributes (foam aggressiveness, acidity, sweetness, bitterness, fruitiness, persistence). This training was carried out selecting carefully several sparkling wines from the market with the proper characteristics to train the tasters in every session. After the training, each candidate to form part of the panel was tested to validate their criteria.

Next, the descriptive sensory analysis was carried out employing high-quality sparkling glasses (Riedel®). The visual sensory analysis was performed in the same sparkling glass to avoid possible differences due to the minimal imperfections of the glass. For each evaluation, 50 mL of sparkling wine at 6–8 °C was served in each glass. The selected attributes were on a tasting-card and panelists were asked to rank each descriptor on a 15-cm unstructured scale (from unnoticeable to very strong).

2.8. Statistical analysis

Statistical analyses were carried out using InfoStat 2017p version (FCA-Universidad Nacional de Córdoba, Argentina). The data were analyzed using the analysis of variance (ANOVA) followed by a Least Significant Difference (LSD-Fisher) test. The LSD test is a post hoc test that determines statistically significant differences between the means with a significance level of 95% ($p < 0.05$). The statistical analyses for sensorial evaluations were analyzed using ANOVA followed by Friedman test ($p < 0.05$). Principal component analysis (PCA) was performed with SPSS software (IBM, Barcelona, Spain).

3. Results and discussion

3.1. Enological parameters

Table 1 shows some general parameters determined in the sparkling wines. Base wine (BW) presented an adequate alcoholic degree and pH for the production of a sparkling wine. The increase of the concentration of ethanol from the BW to the sparkling wine after 12 months of aging (12M) is a natural effect of the second fermentation, increasing by approximately 1% *v*/v. However, we also observed an increase in pH during the aging time. According to the sugar content, this wine belongs to the "Brut" category, following the legal classification of sparkling wines, presenting amounts below 12 $g L^{-1}$ of sugar (Ley, 2011). These results, show that the grapes were healthy and that appropriate winemaking practices were used, thus the enological parameters confirmed that the sparkling wines meet the legal and quality standards, which are within the ranges reported in the literature (Ganss et al., 2011; Martínez-Lapuente, Guadalupe, Ayestarán, Ortega-Heras, & Pérez-Magariño, 2013; Ruiz-Moreno et al., 2017; Torrens, Riu-Aumatell, Vichi, López-Tamames, & Buxaderas, 2010; Zoecklein, 2002). On the other hand, luminosity results (L*) increased during the vinification process and over the period in contact with lees, which was highest for the 6M, 9M, and 12M samples. The a* parameter, which represents the red-green component in the CIELab space, decreased during the production of the sparkling wines. The b* parameter also decreased after 6 months of contact with lees. Despite this, b* had a tendency to increase during the vinification process and aging. Moreover, the color intensity (CI) of the wines tended to decrease, especially following the second fermentation. During aging in contact with lees, the CI tended to decrease, which might be due to adsorption of some polyphenols to the yeast cell wall (Del Barrio-Galán, Pérez-Magariño, Ortega-Heras, Williams, & Doco, 2011; Márquez, Millán, Souquet, & Salmon, 2009). These adsorbed phenolic compounds would precipitate, avoiding its oxidation, being in agreement with the decrease of a* parameter and the increase of L*.

3.2. Volatile compounds determination

Table 2 shows the HS-SPME-GC–MS data. A total of 50 volatile compounds were identified. The most numerous chemical group was the esters (23), followed by alcohols (9), terpenes (7), norisoprenoids (6), acids (3), and aldehydes (2).

As expected, in the must, few esters were detected and in quantities too low to be quantified. After alcoholic fermentation, esters were produced by yeast in reactions between alcohols and acetyl CoA, catalyzed by alcohol acetyltransferase (Mamede, Cardello, & Pastore, 2005) (Fig. 1). The major ester was β-phenethyl acetate, followed by isoamyl acetate, isoamyl octanoate, ethyl octanoate, and ethyl hexanoate. In the process from base to sparkling wine, four esters were detected; ethyl lactate and three tentatively identified as methyl-2-oxo-nonanoate, vinyl decanoate, and diethyl malate. Also, the concentration of five esters increased and 12 compounds of this class decreased during the production of the sparkling wine. The total esters concentration was reduced by approximately half, mainly due to the decrease of β-phenethyl acetate and isoamyl acetate, agreeing with previous results (Riu-Aumatell et al., 2006). The above-mentioned compounds persisted as the major compounds after the second fermentation and after 12 months of contact on lees, but the striking concentration differences detected among the major esters in the base wine disappear. This is due to the different loss rates of each compound after the second fermentation. While β-phenethyl acetate, the major ester in the base wine, decreased by around 90%, ethyl hexanoate reduced by just 25% during the second fermentation. After 12 months of contact with lees, the concentration of almost all the esters decreased or remained stable in the sparkling wine, except ethyl lactate, methyl-2-oxo-nonanoate (t.i), and diethyl succinate. Ethyl lactate and diethyl succinate have been previously reported as markers of the aging time (Francioli et al., 2003; Riu-Aumatell et al., 2006). However, methyl-2-oxo-nonanoate (t.i) was not present in the BW and appeared after the second fermentation, increasing in concentration until 12 months of aging, and, therefore, could also be considered an aging time marker. This compound has

Table 1
Enological and spectrophotometric parameters of sparkling wine during production of sparkling wines.

	M	BW	0M	3M	6M	9M	12M
Brix	18.3	–	–	–	–	–	–
pH	3.08 ± 0.02	2.88 ± 0.02	2.88 ± 0.03^{c}	2.74 ± 0.03^{d}	2.84 ± 0.03^{c}	2.97 ± 0.03^{b}	3.03 ± 0.03^{a}
VA[1]	–	0.09 ± 0.01	0.18 ± 0.05^{a}	0.14 ± 0.02^{a}	0.18 ± 0.02^{a}	0.18 ± 0.01^{a}	0.23 ± 0.00^{a}
RS[2]	187.50	1.44 ± 0.05	8.37 ± 0.79^{ab}	9.67 ± 0.95^{a}	7.74 ± 0.68^{b}	8.78 ± 0.94^{a}	6.40 ± 0.53^{b}
°A[3]	–	10.70 ± 0.21	–	–	–	–	11.73 ± 0.10
CIEL*a*b*[4]							
L*	92.5 ± 0.21	97.50 ± 0.01	97.05 ± 0.35^{c}	97.45 ± 0.21^{bc}	97.85 ± 0.07^{ab}	97.80 ± 0.05^{ab}	98.00 ± 0.05^{a}
C*	12.33 ± 0.08	4.97 ± 0.01	6.80 ± 0.39^{a}	6.55 ± 0.04^{a}	5.74 ± 0.20^{b}	5.80 ± 0.16^{b}	5.38 ± 0.15^{b}
H*	39.73 ± 0.30	63.21 ± 0.02	79.51 ± 2.94^{a}	82.38 ± 1.91^{a}	86.33 ± 4.07^{a}	82.42 ± 0.22^{a}	84.88 ± 1.37^{a}
a*	9.49 ± 0.05	2.24 ± 0.01	1.42 ± 0.17^{a}	1.01 ± 0.01^{b}	0.72 ± 0.11^{c}	0.77 ± 0.01^{c}	0.39 ± 0.01^{d}
b*	7.88 ± 0.10	4.44 ± 0.01	6.68 ± 0.32^{a}	6.49 ± 0.07^{a}	5.72 ± 0.23^{b}	5.75 ± 0.16^{b}	5.35 ± 0.13^{b}
CI[5]	0.34 ± 0.01	0.25 ± 0.01	0.19 ± 0.01^{a}	0.18 ± 0.01^{a}	0.16 ± 0.01^{b}	0.15 ± 0.00^{b}	0.14 ± 0.01^{b}
% Yellow	48.9 ± 0.2	54.7 ± 1.3	63.3 ± 2.4^{a}	63.7 ± 0.2^{a}	64.2 ± 1.4^{a}	64.5 ± 0.2^{a}	66.4 ± 0.5^{a}

Values correspond to averages ± standard deviation (n = 3). Different letters horizontally indicate significant differences at a level of significance of 5%, according to the Fisher LSD test.

[1] VA: Volatile acidity expressed as g $L^{-1}$of acetic acid.
[2] RS: Reducing sugar expressed as g L^{-1} of glucose.
[3] °A: Alcoholic degree in % v/v.
[4] Expressed in CIELAB Units.
[5] CI: Color intensity, expressed in U.A. (Absorbance unit).

been previously identified in Shiraz wine (Chin, Eyres, & Marriott, 2015).

In general, the content of esters decreased during aging (Fig. 1). The typical decreases in acetates ester concentrations observed in sparkling wine (Francioli et al., 2003; Torrens et al., 2010), might be useful as age markers (Riu-Aumatell et al., 2006). However, comparing ethyl and acetate ester losses during this period, the total ethyl esters reduced by 50%, whereas the acetate esters reduced by 85%. It has been demonstrated that lees sorption phenomena affect wine aroma (Gallardo-Chacón et al., 2010; Gallardo-Chacón, Vichi, López-Tamames, & Buxaderas, 2009; Medina Carnicer et al., 2002) and, as a consequence, some volatiles concentrations decrease. More hydrophobic volatile compounds tend to adsorb more easily to the lees, therefore decreasing its presence in the wine. However, additional mechanisms are also likely to be involved because, for example, the hydrophobicity of β-phenethyl acetate (log P = 2.3) (PubChem 2017) and isoamyl acetate (log P = 2.2) are lower than ethyl hexanoate (log P = 2.4), octanoate (log P = 3.5), and decanoate (log P = 4.6), being their losses less pronounced. The decrease of acetate esters might also be due to their chemical hydrolysis due to their thermodinamical unstability and has been observed by other authors (Ruiz-Moreno et al., 2017).

Concerning the alcohols, 3-methyl-1-pentanol, hexanol, decanol, and cis-3-hexenol were determined in the País grape must, with cis-3-hexenol being most predominant. The alcohols augmented after alcoholic fermentation, and the total concentration remained almost constant after the second fermentation and throughout aging (Fig. 1). It has been postulated that yeast lees seem to possess a scarce capacity to retain alcohols on their surface (Gallardo-Chacón et al., 2009). On the other hand, contrary to the results obtained by other authors (Riu-Aumatell et al., 2006; Torrens et al., 2010), in this case, 2-phenylethanol did not change significantly after 12 months of contact with lees. However, hexanol had the same tendency to increase as reported in the above-cited studies, therefore being a suitable aging marker.

The same trend as the alcohols was observed in the acids. Three acids were found in the samples: hexanoic, octanoic, and decanoic acids. In the must, only hexanoic and decanoic acid were detected and, after alcoholic fermentation, remained unchanged until the end of the sparkling wine production process (12M).

For the aldehydes, hexanal was found in the must, but its concentration decreased naturally transforming in the corresponding derived alcohols during alcoholic fermentation (Fig. 1). Furfural appeared after the second fermentation (0M) and, after 12 months of contact with lees (12M samples), its concentration had doubled. This compound is a furan derived by sugar degradation that increases during the aging process (Torrens et al., 2010).

In addition to the above-mentioned compounds, we identified or tentatively identified several compounds that are typically bounded to sugars in the grape and wine. These glycosylated compounds are released by yeast enzymes (Riu-Aumatell et al., 2006) or from these nonvolatile precursors by hydrolysis under acidic conditions at wine pH (Williams, Strauss, Wilson, & Massy-Westropp, 1982). This is the case of norisoprenoids, which were scarcely detected in the must in their free form, with β-damascenone being the major form found before alcoholic fermentation. However, during alcoholic fermentation, several volatile compounds were released by yeast, significantly increasing their presence in the BW. Seven norisoprenoids were semi-quantified (relative area), with β-damascenone consistently present at the highest concentration. After the second fermentation, we detected a decrease in the concentration of some norisoprenoids, such as α-ionene (t.i), ionone (t.i), and β-damascenone. The decrease in the concentration of β-damascenone during wine production has been previously reported by Torrens et al. (2010), proposing that some mannoproteins released from the yeast might interact with this compound and cause this change, as reported by Chalier, Angot, Delteil, Doco, and Gunata (2007). However, other authors have reported an increase in the concentration of this compound after the second fermentation (Ganss et al., 2011). Conversely, vitispirane 1 (t.i) and TDN (t.i) increased after the second fermentation. These are carotenoid-derived megastigmane compounds and typically increase during the winemaking process (Riu-Aumatell., 2006; Torrens et al., 2010) because of the direct degradation of a carotene or from aroma precursors linked to a sugar molecule. After 12 months in contact with lees, ionone, vitispirane 1, and vitispirane 2 (t.i) concentrations increased significantly, whereas the concentrations of α-ionene and β-damascenone decreased. Surprisingly, the TDN concentration remained stable during the 12 months of aging, which is contrary to the results reported by Bosch-Fusté et al. (2007) and Torrens et al. (2010) after 14 months of aging.

Terpenes are another group of glycosylated compounds that might be present in the grape and wine. Nerolidol, Linalool, and α-terpineol were identified in the País grape must, with the latter two compounds previously described among the aromatic precursors of this variety (Ubeda, del Barrio-Galán, Gil I Cortiella, & Peña-Neira, 2017b). Similarly, as occurred with norisoprenoids, terpenes increased their concentration after alcoholic fermentation. Also, some compounds that

Table 2
Volatile compounds from the must (M), base wine (BW) and sparkling wines with different months of contact with lees: 0 months (0M), 3 months (3M), 6 months (6M), 9 months (9M) and 12 months (12M).

Compounds	LRI	ID	M	BW	0M	3M	6M	9M	12M
Esters									
Ethyl butanoate	1055	A	n.d.	1041 ± 35	462 ± 24^{ab}	594 ± 67^{bc}	667 ± 52^{c}	662 ± 67^{c}	450 ± 127^{ab}
Ethyl 2 methylbutanoate	1084	A	n.q.	3.63 ± 0.11	22.7 ± 3.8^{d}	10.6 ± 1.6^{a}	13.4 ± 0.7^{ab}	13.8 ± 1.1^{ab}	17.2 ± 3.8^{bc}
Ethyl isovalerate	1098	A	n.d.	n.d.	6.35 ± 0.81^{b}	2.48 ± 0.98^{a}	4.86 ± 0.85^{b}	2.08 ± 1.30^{a}	3.71 ± 0.92^{ab}
Ethyl hexanoate	1245	A	n.d.	1471 ± 29	622 ± 29^{a}	865 ± 149^{a}	802 ± 111^{a}	620 ± 181^{a}	670 ± 152^{a}
Ethyl heptanoate[1]	1334	B	n.q.	32.1 ± 5.6	37.8 ± 1.3^{a}	40.9 ± 0.9^{a}	39.1 ± 2.1^{a}	45.5 ± 2.2^{a}	40.1 ± 7.5^{a}
Ethyl lactate	1379	A	n.d.	n.d.	3.12 ± 0.31^{a}	4.83 ± 2.52^{a}	16.9 ± 3.2^{b}	19.9 ± 4.2^{b}	48.9 ± 9.1^{c}
Methyl octanoate	1401	A	n.d.	13.1 ± 0.1	9.22 ± 0.23^{d}	5.29 ± 0.79^{bc}	4.33 ± 0.71^{ab}	4.46 ± 1.11^{abc}	3.86 ± 0.72^{a}
Ethyl octanoate*	1437	A	n.d.	3.31 ± 0.04	1.69 ± 0.05^{a}	1.71 ± 0.34^{a}	1.43 ± 0.36^{a}	1.49 ± 0.32^{a}	1.21 ± 0.21^{a}
Methyl 2-oxo-nonanoate[3]	1545	B	n.d.	n.d.	74.5 ± 4,1^{c}	65.5 ± 8.2^{bc}	48.9 ± 4.1^{ab}	108 ± 13^{d}	109 ± 20^{d}
Ethyl nonanoate[1]	1558	B	n.d.	14.1 ± 0.8	54.5 ± 1.6^{e}	7.86 ± 1.73^{b}	4.13 ± 1.11^{a}	11.4 ± 0.9^{c}	7.09 ± 1.14^{b}
Ethyl decanoate	1647	A	n.d.	1267 ± 29	605 ± 22^{c}	533 ± 128^{c}	311 ± 90^{ab}	470 ± 72^{bc}	272 ± 98^{a}
Diethyl succinate	1675	A	n.q.	55.3 ± 2.5	256 ± 8^{ab}	301 ± 22^{bc}	350 ± 20^{c}	423 ± 39^{d}	540 ± 47^{e}
Isoamyl octanoate*	1680	A	n.d.	6.48 ± 0.37	3.33 ± 0.10^{c}	2.20 ± 0.72^{b}	1.68 ± 0.24^{ab}	1.98 ± 0.37^{ab}	1.36 ± 0.15^{a}
Ethyl trans-4-decenoate[1]	1704	C	n.d.	15.5 ± 1.1	325 ± 11^{c}	227 ± 49^{b}	217 ± 27^{b}	204 ± 17^{b}	111 ± 28^{a}
Vinyl decanoate[2]	1747	C	n.d.	n.d.	113 ± 4^{c}	95.9 ± 14.6^{bc}	61.7 ± 15.3^{a}	146 ± 10^{d}	95.7 ± 18.6^{bc}
Ethyl dodecanoate[1]	1864	B	n.q.	693 ± 77	42.4 ± 2.9^{ab}	57.8 ± 14.2^{bc}	32.2 ± 8.1^{a}	34.3 ± 3.9^{a}	40.5 ± 10.5^{ab}
Isoamyl decanoate	1888	A	n.d.	971 ± 196	353 ± 62^{b}	251 ± 56^{ab}	166 ± 35^{a}	160 ± 24^{a}	159 ± 33^{a}
Isobutyl octanoate[3]	1905	C	n.d.	102 ± 5	19.8 ± 1.3^{b}	n.d.	n.d.	n.d.	n.d.
Ethyl tetradecanoate[1]	2041	B	n.d.	6.38 ± 0.73	5.06 ± 0.88^{b}	3.11 ± 0.81^{a}	2.18 ± 0.44^{a}	2.60 ± 0.37^{a}	3.42 ± 0.34^{ab}
Diethyl malate[4]	2057	B	n.d.	n.d.	54.2 ± 18.1^{a}	45.4 ± 7.1^{a}	53.2 ± 12.9^{a}	61.7 ± 14.6^{a}	79.2 ± 36.8^{a}
Total sum ethyl esters*			–	15.5	8.06	6.96	5.92	6.48	5.27
Isoamyl acetate*	1122	A	n.d.	7.31 ± 0.18	2.27 ± 0.42^{c}	2.64 ± 0.44^{c}	2.21 ± 0.17^{bc}	1.57 ± 0.46^{ab}	1.08 ± 0.31^{a}
Hexyl acetate	1285	A	n.q.	521 ± 10	121 ± 4^{bc}	124 ± 15^{bc}	98.8 ± 8.8^{b}	51.8 ± 13.6^{a}	49.6 ± 12.4^{a}
β-phenylethyl acetate*	1860	A	n.d.	8.02 ± 0.33	2.15 ± 0.07^{c}	2.18 ± 0.26^{c}	1.54 ± 0.17^{b}	1.12 ± 0.21^{a}	0.819 ± 0.223^{a}
Total sum acetate esters*			–	15.8	4.54	4.94	3.84	2.74	1.94
Alcohols									
Propanol*	1049	B	n.q.	8.98 ± 0.35	5.93 ± 0.13^{a}	9.67 ± 1.57^{cd}	7.68 ± 1.04^{ab}	10.1 ± 1.1^{d}	8.11 ± 1.09^{bc}
Isobutanol*	1074	A	n.d.	19.2 ± 1.1	15.9 ± 0.5^{b}	17.8 ± 0.9^{b}	16.3 ± 1.9^{b}	16.5 ± 4.4^{b}	4.63 ± 1.09^{a}
Isoamyl alcohol*	1200	A	n.d.	91.9 ± 2.5	86.8 ± 2.7^{a}	101 ± 5^{a}	88.4 ± 8.5^{a}	101 ± 5^{a}	101 ± 18^{a}
3-methyl-1-pentanol	1332	B	48.2 ± 7.4	76.9 ± 17.5	103 ± 4^{a}	109 ± 5^{a}	101 ± 5^{a}	110 ± 5^{a}	120 ± 16^{a}
Hexanol*	1375	A	0.273 ± 0.156	2.75 ± 0.11	3.05 ± 0.12^{a}	3.30 ± 0.09^{ab}	3.15 ± 0.16^{a}	3.64 ± 0.19^{b}	3.65 ± 0.41^{b}
cis-3-hexenol	1410	A	441 ± 28	239 ± 17	225 ± 5^{a}	244 ± 32^{a}	268 ± 12^{a}	317 ± 39^{b}	322 ± 34^{b}
2,3-butanediol	1534	A	n.d.	434 ± 32	394 ± 90^{a}	798 ± 44^{b}	489 ± 107^{a}	783 ± 74^{b}	806 ± 167^{b}
Decanol	1773	A	0.484 ± 0.001	5.07 ± 0.67	5.78 ± 0.24^{a}	6.38 ± 0.48^{a}	6.24 ± 0.62^{a}	5.51 ± 0.52^{a}	5.51 ± 0.12^{a}
β-phenylethanol*	1940	A	n.d.	11.38 ± 1.03	11.6 ± 0.6^{a}	11.8 ± 0.9^{a}	10.6 ± 0.1^{a}	10.9 ± 0.9^{a}	12.7 ± 0.3^{a}
Aldehydes									
Hexanal	1083	A	366 ± 6	12.1 ± 0.1	24.7 ± 2^{b}	12.5 ± 0.1^{a}	12.6 ± 0.2^{a}	12.3 ± 0.1^{a}	12.1 ± 0.1^{a}
Furfural	1438	A	n.d.	n.d.	26.2 ± 0.9^{b}	20.6 ± 1.1^{b}	11.3 ± 2.6^{a}	25.1 ± 4.4^{b}	40.1 ± 10.9^{c}
Norisoprenoids									
α-ionene	1510	C	0.0793 ± 0.0055	8.03 ± 0.34	0.893 ± 0.052^{d}	0.747 ± 0.135^{c}	0.429 ± 0.081^{b}	0.404 ± 0.019^{b}	0.235 ± 0.031^{a}
Vitispirane 1	1518	A	0.0643 ± 0.0136	3.81 ± 0.41	9.53 ± 0.25^{a}	12.5 ± 1.5^{b}	12.2 ± 1.4^{b}	13.5 ± 1.9^{b}	14.6 ± 2.1^{b}
Vitispirane 2	1522	A	0.186 ± 0.035	9.72 ± 1.12	9.23 ± 0.34^{a}	11.8 ± 1.3^{b}	10.7 ± 1.4^{ab}	11.9 ± 1.4^{b}	11.7 ± 2.1^{b}
TDN	1745	A	0.149 ± 0.021	8.99 ± 0.35	9.48 ± 0.29^{a}	8.34 ± 1.39^{a}	7.91 ± 2.17^{a}	9.89 ± 3.97^{a}	10.4 ± 1.1^{a}
β-damascenone	1849	A	4.28 ± 0.34	17.8 ± 0.7	10.1 ± 0.4^{e}	9.15 ± 0.89^{cd}	7.98 ± 0.56^{ab}	8.44 ± 0.51^{bc}	7.47 ± 0.44^{a}
Ionone	1931	A	0.121 ± 0.026	7.66 ± 0.79	4.06 ± 0.16^{a}	12.2 ± 1.8^{c}	11.7 ± 1.3^{c}	12.7 ± 1.6^{c}	7.19 ± 1.07^{b}
Terpenes									
Linalool formate[5]	1501	C	n.d.	3.23 ± 0.04	4.26 ± 0.53^{b}	3.78 ± 0.42^{b}	3.76 ± 0.25^{b}	4.06 ± 0.61^{b}	1.78 ± 0.24^{a}
Linalool	1555	A	0.261 ± 0.071	2.89 ± 0.11	2.31 ± 0.13^{bc}	2.07 ± 0.41^{b}	1.78 ± 0.21^{ab}	1.38 ± 0.46^{a}	1.29 ± 0.39^{a}
Hotrienol[6]	1670	B	n.d.	2.06 ± 0.12	1.73 ± 0.12^{c}	1.18 ± 0.18^{abc}	0.94 ± 0.04 ^{ab}	0.459 ± 0.906^{a}	1.42 ± 0.14^{bc}
Citronellol acetate[5]	1685	B	n.d.	19.8 ± 1.2	4.59 ± 0.37^{b}	4.35 ± 0.78^{b}	3.21 ± 0.67^{b}	0.231 ± 0.534^{a}	0.958 ± 0.432^{a}
α-terpineol	1693	A	0.906 ± 0.052	2.08 ± 0.15	4.33 ± 0.11^{ab}	4.45 ± 0.67^{abc}	4.88 ± 0.14^{bc}	5.27 ± 0.96^{bc}	5.40 ± 0.64^{c}
Citronellol	1785	A	n.d.	2.84 ± 0.09	1.81 ± 0.15^{c}	1.83 ± 0.33^{c}	1.32 ± 0.16^{b}	0.707 ± 0.235^{a}	0.772 ± 0.237^{a}
Nerolidol	2056	A	0.789 ± 0.004	7.03 ± 0.13	9.94 ± 0.87^{c}	8.01 ± 1.12^{b}	6.95 ± 1.21^{b}	4.05 ± 0.68^{a}	3.26 ± 0.63^{a}
Acids									
Hexanoic acid*	1880	A	0.0589 ± 0.0097	6.16 ± 0.44	7.27 ± 0.31^{a}	8.24 ± 0.54^{a}	7.78 ± 0.45^{a}	8.52 ± 0.81^{a}	8.42 ± 0.11^{a}
Octanoic acid*	2076	A	n.d.	6.73 ± 0.38	7.46 ± 0.31^{a}	7.81 ± 0.75^{a}	7.35 ± 0.73^{a}	7.44 ± 0.65^{a}	8.23 ± 1.34^{a}
Decanoic acid*	2329	A	0.0564 ± 0.0021	1.47 ± 0.12	1.23 ± 0.07^{a}	1.15 ± 0.81^{a}	1.14 ± 0.11^{a}	1.09 ± 0.11^{a}	1.29 ± 0.09^{a}

Results (average ± SD) are expressed as μg L^{-1} except those marked with an asterisk (*), which are expressed in mgL^{-1}. C_{13}-Norisoprenoids are expressed in relative area.
Values with different superscript letter indicate statistically significant differences ($p < 0.05$).
ID: reliability of identification: A, mass spectrum and LRI agreed with standards; B, mass spectrum agreed with mass spectral data base and LRI agreed with the literature data; C, tentatively identified, mass spectrum agreed with mass spectral database.
LRI: Linear Retention Index; n.d.: not detected; n.q.: not quantifiable.
[1] Undecaonate equivalents.
[2] Isobutyl acetate equivalents.
[3] Ethyl-2-methylbutyrate equivalents.
[4] Ethyl butyrate equivalents.
[5] Citronellol equivalents.
[6] Linalool equivalents.

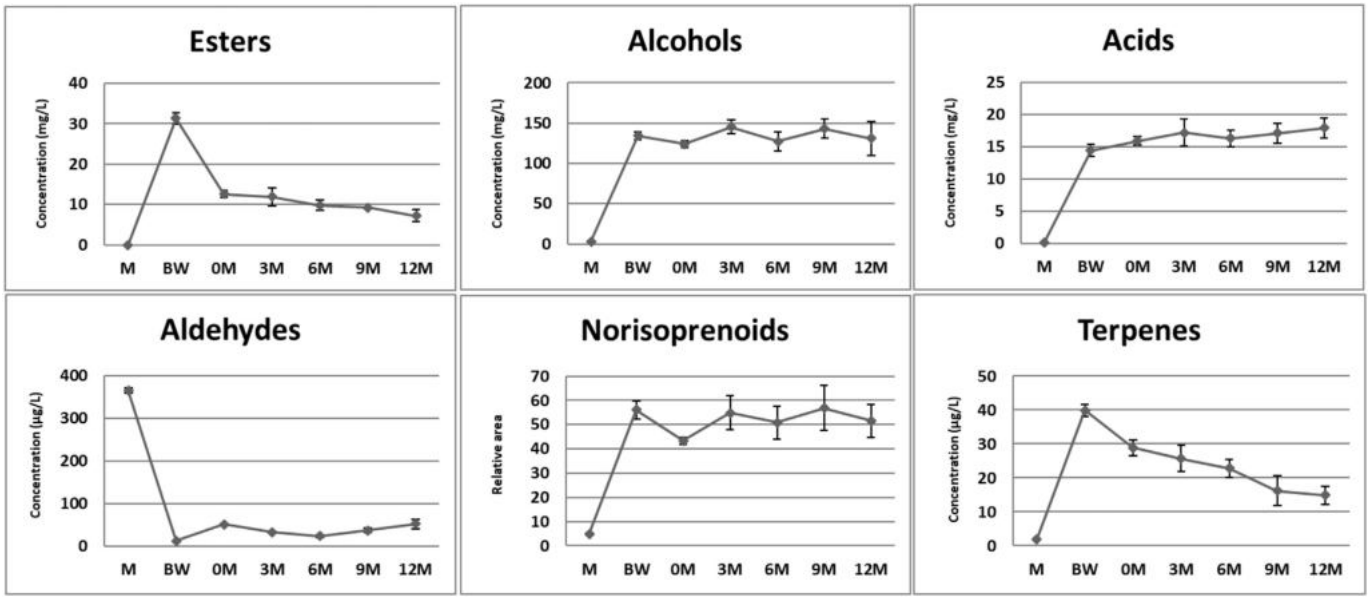

Fig. 1. Evolution of the different chemical families of volatile compounds during the production of sparkling wine. M (Must); BW (Base wine); 0M, 3M, 6M, 9M, 12M (Sparkling wine of different months in contact with lees).

were not present in the must appear, such as linalool formate (t.i), citronellol, hotrienol (t.i), and citronellol acetate (t.i). The second fermentation resulted in a slight reduction in the concentration of terpenes, which is in contrast with the findings of Ganss et al. (2011), who reported an increase in the concentration of terpenes. During the aging on lees, all terpenes decreased, except hotrienol (t.i) (unchanged) and α-terpineol (increased). After 12 months on lees, α-terpineol replaced nerolidol as the most abundant terpene.

3.3. Olfactometry analysis

For this study, it has been only considered odor active those odor zones which were perceived in at least three times of the total sniffing trials (12 per sample). The odor-active compounds are listed in Table 3; some have only been tentatively identified, whereas others have not been identified. By GC-O analyses 48, 47 and 38 odor-active zones were detected in the BW, sparkling wine after second fermentation (0M), and sparkling wine after 12 months of aging (12M), respectively. Among these, 23 odor zones were perceived by the panellist in the three samples (BW, 0M and 12M). The odor zones with the highest modified frequency (MF) from BW to 12M were ethyl isobutyrate, ethyl hexanoate, and isoamyl acetate, which are described as fruity, anise, and strawberry and banana aromas respectively being possible impact aromas in País grape variety sparkling wine. However, Campo et al. (2005), described 4 odorants with MF above 75% in cava: ethyl hexanoate, isoamyl alcohol, ethyl-3-methylbutyrate and ethyl-3-methylbutyrate, being ethyl hexanoate and isoamyl alcohol in agreement with our findings. In the volatile compounds analysis, some odor zones associated to esters appeared after the second fermentation, decreasing from 31 to 29 odor zones, and to 22 odor zones in the sparkling wine after 12 months of contact with lees. These zones were described as fruity/floral aromas. This reinforces the previously described loss of freshness of the sparkling wines after an aging period (Bosch-Fusté et al., 2007; Torrens et al., 2010).

Seven aromatic zones associated with terpenes were perceived in the base wine, whereas six were detected in the sparkling wines, all of which were related to citric/floral/herb aromas. Also, the MF in the zones perceived in the BW was higher than in the sparkling wines. This finding is consistent with the decrease observed in the concentration of the volatile compounds after the second fermentation and during aging.

The odor zones related to diethyl succinate (fermented/floral/lactic), ethyl lactate (fruity/spiced), and ethyl isovalerate increased after the second fermentation (0M) and aging (12M), which was consistent with our GC–MS analysis and the findings of other authors (Torrens et al., 2010).

On the other hand, TDN, an important marker of aging and described as burned/tabac/herb, did not experience significant concentration changes during aging and was only perceived in the sparkling wines. Also, after 12 months of contact with lees, the panelists could perceive an aromatic zone associated with vitispirane, another important marker of aging time.

Fig. 2 shows the contribution of each aroma category as a percentage of the total MF of odor zones. This figure was built first dividing the odor active compounds into five categories: Fruity, floral, citric, vegetable and miscellaneous. Then it was related the sum of the values of MF of each odor category in each sample with the total sum of the values of the values of MF of the entire number of aroma compounds in that sample (100%). In contrast to the GC–MS analysis results, the zones described as fruity aromas were similar when comparing the BW (39% of the total MF) and sparkling wine after the second fermentation (40% of the total MF). However, a decrease of the total MF was observed after the 12 months of contact with lees (36%), supporting the loss of fruity nuances after aging, which is linked to the decrease of esters. Also, aromatic floral zones experienced a decrease in the frequency of detection and intensity from the BW to the final sparkling wine. The zones associated with a group of miscellaneous aromas increased their MF total percentage during the production process, reaching a maximum after aging. This might be due to the bound aroma compounds released by yeast enzymes, such as vitispirane and TDN, which are included in this group.

3.4. Sensory analysis

Table 4 shows data from the visual, aromatic and gustative sensorial analysis of the sparkling wines. We found that color intensity and the red component decrease during aging. These results agree with a higher a* component (red component intensity) and CI (Table 1). These findings are in line with those of Martínez-Lapuente et al. (2013), who compared sparkling wines with 9 months of aging on lees and found a strong correlation between the color perception and the instrumental

Table 3
Odor-active compounds in base and sparkling wines.

LRI	ID	Odorant	MF			Odor descriptor
			BW	0M	12M	
<800	t.i	Methyl butyrate	–	29	47	fruity
<800	A	Isoamyl alcohol	76	69	75	fruity/glue/yeast
<800	A	Ethyl isobutyrate	85	88	83	strawberry
<800	–	unknown	50	37	44	plastic/ burned
807	A	Ethyl butyrate	82	73	62	fruity
822	A	Ethyl lactate	37	41	60	fruity/spiced
849	A	Ethyl 2-methylbutyrate	65	83	58	citric fruit/watermelon
856	A	Ethyl isovalerate	58	47	82	fruity/pineapple
881	t.i	3-Ethyl-1-butanol	37	29	–	vegetable/herb
891	A	Hexanol	87	71	80	cooked vegetables/ yeast
912	A	Isoamyl acetate	88	90	91	banana
930	t.i	methoxy-phenyl-oxime	71	85	75	cooked potatoes/yeast
955	–	unknown	29	58	53	citric/green
978	–	unknown	41	50	55	floral
993	t.i	3-methylpentyl acetate	44	–	–	fruity/caramel
1008	A	Ethyl hexanoate	93	94	96	anise/strawberry/ fruity
1013	A	Hexyl acetate	80	55	55	citric fruit
1031	–	unknown	55	–	–	fresh/floral
1047	A	2-Ethyl-1-hexanol	33	41	–	floral/vegetable
1056	–	unknown	53	–	–	floral
1068	A	Isoamyl butanoate	–	53	58	floral
1073	A	Hexanoic acid	47	62	58	rubber/ferric
1077	t.i	Ethyl 2-hexenoate	44	24	37	floral/rose
1090	A	Octanol	–	33	62	solvent
1099	t.i	Ethyl 2-furoate	–	62	–	white flowers
1104	–	unknown	47	–	–	floral/muddy
1119	A	Linalool	44	41	41	citric/floral
1128	A	β-Phenylethanol	58	69	73	floral/rose
1135	A	Nonanol	65	53	50	resin/vegetable
1180	A	Nonanal	41	67	73	ferric/match
1197	t.i	Nerol oxide	60	67	62	floral/rose
1205	t.i	trans-2-pinalol	44	–	–	floral
1208	A	Diethyl succinate	47	47	71	Fermented/ floral/ lactic
1210	A	Octanoic acid	58	44	–	muddy/cooked vegetables
1215	A	Ethyl octanoate	53	53	44	fruity/floral
1223	A	Decanal	–	75	37	Pepper/vegetables
1249	t.i	2-carene	41	24	60	herb/pepper
1266	A	Ethyl phenylacetate	53	44	–	fruity
1279	A	Isoamyl hexanoate	44	47	–	fruity
1283	A	β-Phenethylacetate	–	47	47	floral
1305	t.i	Vitispirane	–	–	37	caramel/spyced
1339	A	Methyl decanoate	33	33	–	fruity
1362	t.i	Ethyl nonanoate	–	47	–	fruity/floral
1379	t.i	α-ionene	47	–	41	citric/solvent
1391	t.i	Isobutyl octanoate	55	53	–	white flowers/anise/ herb
1416	t.i	Citronellol acetate	62	29	29	citric/herb/geranium
1433	A	Ethyl decanoate	23	37	37	citric/herb
1446	t.i	TDN	–	33	47	burned/tabac/herb
1455	t.i	ethyl 3-methylbutyl butanoate	23	33	–	burned/herb
1492	A	Isoamyl octanoate	–	–	33	fruity
1498	t.i	(*E*)-β-Famesene	37	–	–	muddy
1505	–	unknown	47	–	50	toast
1515	t.i	α-Ionone	47	62	–	roasted apple
1613	A	*E*-Nerolidol	–	33	37	floral/citric
1624	t.i	Ethyl dodecanoate	25	24	–	fruity
1651	A	Isoamyl decanoate	25	29	–	floral
1667	t.i	gamma-eudesmol	58	–	–	floral
1691	t.i	Ethyl tetradecanoate	33	–	–	floral/caramel
1754	t.i	Farnesol acetate	47	41	44	floral/apple/jam

LRI: Linear retention index.
MF: Modified frequency.
A: mass spectrum and LRI agreed with standards.
t.i: tentatively identified, mass spectrum agreed with mass spectral database.

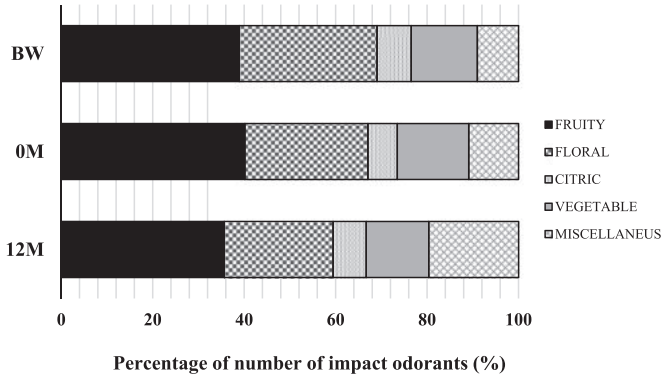

Fig. 2. The contribution of each aroma category as percentage of the number of odor active compounds in Base wine (BW), sparkling wine of 0 (0M) and 12 months in contact with lees (12M).

Table 4
Sensorial attributes analyzed in sparkling wines.

	0M	3M	6M	9M	12M
Visual attributes					
Foamability	a	a	a	a	a
Bubble rate	a	abc	c	ab	abc
Bubble size	a	a	a	a	a
Color intensity	d	d	a	abc	ab
Red component	e	d	bc	ab	a
Foam stability	d	cd	a	c	b
Olfactory attributes					
Aromatic intensity	2.53^{a}	3.22^{a}	2.69^{a}	3.59^{a}	2.97^{a}
Fruity	2.63^{a}	3.28^{a}	3.22^{a}	3.19^{a}	2.69^{a}
Floral	2.03^{a}	3.38^{b}	3.66^{b}	3.16^{b}	2.78^{ab}
Bread/toasted/yeast	2.78^{abc}	2.38^{a}	2.50^{ab}	4.03^{d}	3.31^{abcd}
Complexity	2.53^{a}	3.16^{a}	2.69^{a}	3.56^{a}	3.06^{a}
In mouth attributes					
Foam aggressiveness	3.47^{bcd}	3.97^{d}	2.44^{a}	2.53^{ab}	2.59^{abc}
Acidity	2.84^{a}	3.13^{a}	2.94^{a}	3.28^{a}	2.81^{a}
Sweetness	3.38^{a}	3.59^{a}	3.06^{a}	2.59^{a}	2.38^{a}
Bitterness	3.41^{a}	2.88^{a}	3.50^{a}	2.41^{a}	2.81^{a}
Fruitiness	3.19^{a}	3.63^{a}	2.66^{a}	3.06^{a}	2.47^{a}
Persistence	3.41^{a}	2.97^{a}	2.75^{a}	3.06^{a}	2.81^{a}

The values correspond to the average of the tasting of 16 evaluators (n = 16). Different letters horizontally indicate significant differences at a level of significance of 5% ($p < 0.05$). Friedman's test.

measurement. We detected no significant differences in foamability, crown persistence, and bubble speed and size between the various on-lees aging times. This was probably due to the short period of aging, longer aging periods could result in significant differences in foam characteristics as described in the literature (Kemp et al., 2018). However, the non-aged sparkling wine had a higher effervescence than the aged wines.

The sensory aroma analysis showed that bakery/toasty/yeast nuances were perceived as more intense in the sparkling wines with higher aging time (9M and 12M) compared to less aged sparkling wines. These results agree with Vannier, Brun, and Feinberg (1999), who detected an aromatic evolution during aging to ripe fruits and toasted notes. These bakery/toasty/yeast nuances are typical aromas of sparkling wines that have been in contact with lees and typically increase with aging time. In contrast, Torrens et al. (2010) described floral, fruity, sweet, toast, lactic and yeasty nuances as becoming more complex during the aging of sparkling wines. However, our panelists did not perceive clear differences in the fruitiness, aromatic intensity or complexity of the tested wines. This could be because the highest impact aroma compounds detected in olfactometry analyses were described as fruity aromas (ethyl isobutyrate, ethyl hexanoate, and isoamyl acetate) and accounted for a similar percentage of the MF

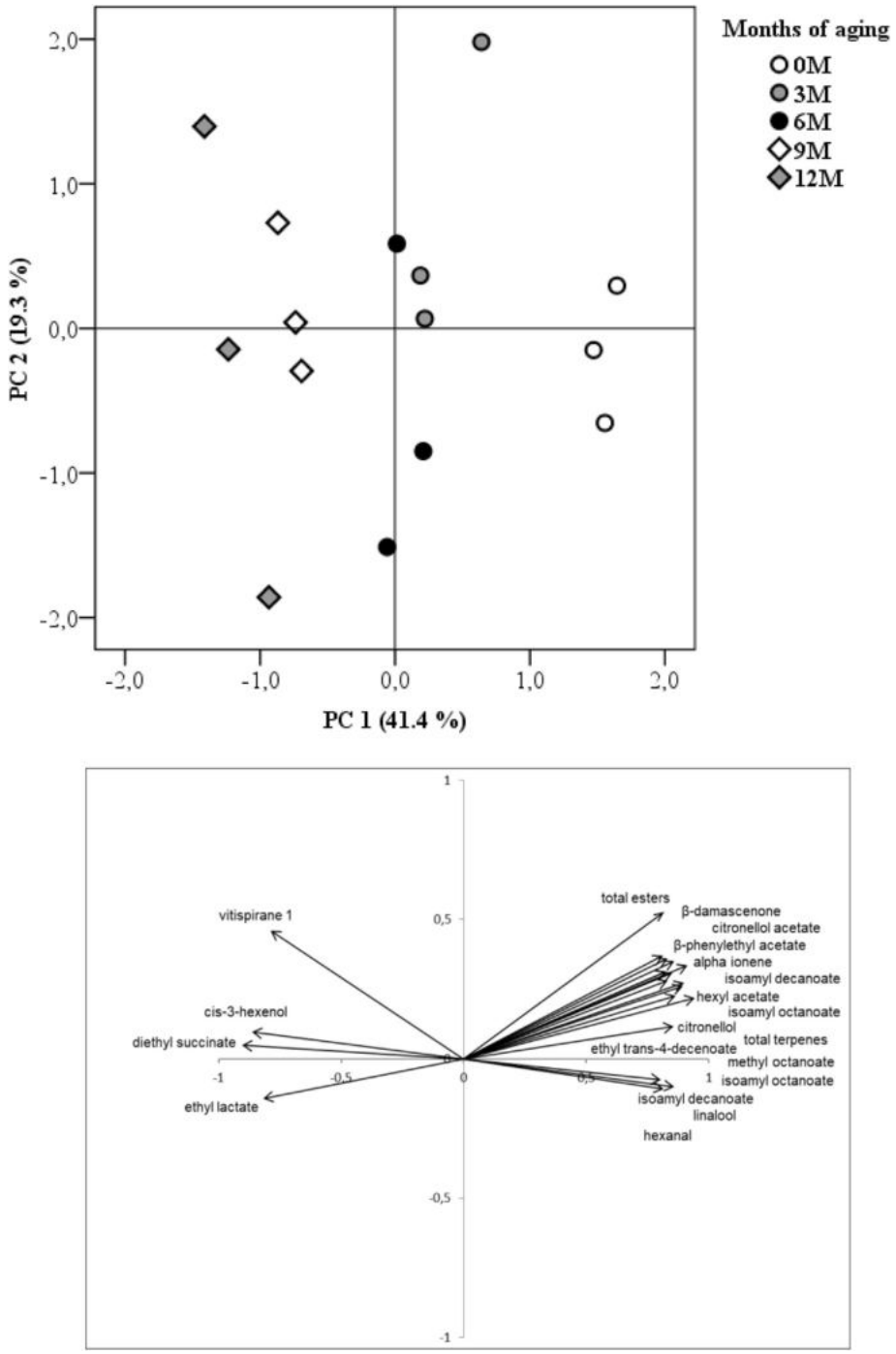

Fig. 3. Data scores and loadings (absolute eigenvalue >0.800) biplot on the plane made up of the first two principal components (PC1 against PC2).

throughout the aging period. In contrast to the GC–MS and GC-O analyses, the floral attribute was perceived as more intense in the 3M, 6M, 9M and 12M samples compared to 0M. This contrast with the fact that floral odor zones perceived during olfactometry analyses decreased during sparkling wine production. This suggests that the floral character of these sparkling wines may be defined by only a few of the determined compounds. This could be the cases of β-phenylethanol and diethyl succinate, witch aromatic zones were perceived with higher intensity in the 12M sample than in the 0M sample. Also, the percent MF of these compounds are correlated with analytical and sensorial data. This might identify β-phenylethanol and diethyl succinate as high-impact volatile compounds and main responsible for the floral attributes of sparkling wine.

Finally, considering the taste aspects (Table 4), the only significant difference was found in the attribute foam aggressiveness, which decreased in the 6M, 9M, and 12M samples relative to the less aged samples (0M and 3M).

3.5. Multivariate analysis

Due to the huge amount of data generated throughout this study, a Principal component analysis (PCA) was performed using all the volatile compounds and the total sum of each chemical group showed in Table 1 (57 variables), in order to allow a dimension reduction and achieve a better understanding of the whole obtained results. All the sparkling wine samples were used to perform the PCA. The analysis

gives six principal components (PCs) with an Eigenvalue > 1, which explained 94.4% of the total variance. Fig. 3 shows the distribution of the analyzed samples (scores) depicted in function of their aging time (months of contact with lees) for PC1 and PC2, which accounted for 41.1% and 19.3% of variance respectively (60.7% of the cumulative variance). Moreover, Fig. 3 also shows the factor values (loadings) for variables with greatest weight in PC1 and PC2. As could be observed in Fig. 3, it seems that samples were distributed among the x-axis (PC1) in function of their aging time; the lower the aging time the higher the PC1 score of the samples. Hence, wines without aging on lees (0M) are grouped at the right side of the graphic and they are well separated from the samples that have had contact with lees. It is not possible to distinguish between the 3M and 6M samples, grouped in the center of the graphic, as also occurs with samples 9M and 12M, grouped in the left side of the graphic. On the other hand, the second component (PC2) does not seem to be related with the wine aging on lees, since scores distribution in the y-axis shows high dispersion and samples were not grouped in function of their aging times. Thus, Component 1 seems to explain the variance among wines with different aging time. According to the graphic of loadings, it seems that throughout time in contact with lees a diminution of several esters took place, along with the loss of terpene compounds and some carbonyl compounds (β-damascenone, α-ionene, hexanal). Since these compounds mainly come from grapes or from grape precursors (especially in the case of esters, formed by the union between alcohols and a carboxylic acids), it is quite logical their loss during wine aging. In contrast, it seems that diethyl succinate, ethyl lactate, *cis*-3-hexenol and vitispirane 1 increase throughout wine aging. These compounds seem to be released along the period of lees contact, and they could be good candidates to become markers of aging on lees for sparkling wines, especially in the case of diethyl succinate, which shows the greatest loading (−0.906) among them.

4. Conclusions

The combination of different analysis techniques allowed having a fairly broad view of the changes that take place in the sparkling wine during its production. There is an important loss of esters during the second fermentation and posterior aging period, which is related to a decrease in the contribution of the odoriferous zones related to fruity aromas. This loss might be due to adsorption onto lees, but also due to the chemical hydrolysis due to their thermodinamical unstability. Despite of this, it was observed that diethyl succinate increased during aging and could be one proper aging marker.

Several compounds increased their concentration during aging, and therefore could be used as aging markers (e.g., norisoprenoids). Based on our findings, we propose that, for young sparkling wines (12 months in contact with lees), vitispiranes might be better aging markers than the typically used TDN.

The fruit and floral odor zones diminished during aging. However, this was not perceived in the sensorial trials. This suggests that the responsibility for fruity and floral nuances in sparkling wine resides in a few high-impact aromatic compounds that exhibit this behavior in olfactometry and mass spectral quantification data, such as ethyl isobutyrate, isoamyl acetate, ethyl hexanoate, and β-phenylethanol. In addition, aging had a strong influence on the color of the sparkling wines, mainly due to the adsorption of pigment molecules to the lees cellular wall, which was perceived by the tasting panel. Nevertheless, 12 months in contact with lees was insufficient for the panel to perceive more remarkable differences such as changes in the fruity nuances perception, foamability, etc.

Finally, based on our findings, we propose that País grape is a suitable variety for the production of sparkling wines, giving rise to a sparkling wine characterized by a marked floral character.

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