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Comparative study of volatile substances and ellagitannins released into wine by *Quercus pyrenaica*, *Quercus petraea* and *Quercus alba* barrels

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ABSTRACT

The aim of this work was to study the cooperage potential of the oak species *Quercus pyrenaica*, which is widespread throughout the Iberian Peninsula. A red wine of 2016 vintage was aged in new barrels made from *Quercus pyrenaica*, *Quercus petraea* and *Quercus alba* for 12 months. This process was repeated with a similar red wine from the subsequent vintage using the same barrels in order to compare the performances of the new *Q. pyrenaica* barrels and *Q. pyrenaica* barrels that had been used for one year with equivalent *Q. petraea* and *Q. alba* barrels. The results indicate that *Q. pyrenaica* releases levels of β -methyl- γ -octalactone similar to those released by *Q. alba* and clearly higher than *Q. petraea*, whereas it releases levels of ellagitannins similar to *Q. petraea* and clearly higher than *Q. alba*. These data indicate that *Q. pyrenaica* is more similar to American oak from an aromatic point of view, since it provides mainly coconut notes, but is more similar to French oak in terms of wine structuration. Moreover, based on the preferences of a trained panel, the wines aged in the *Q. pyrenaica* barrels were ranked in second position, just behind the wines aged in the *Q. petraea* barrels and ahead of those aged in the *Q. alba* barrels. Consequently, *Q. pyrenaica* seems to have characteristics midway between the two most commonly used oak species for cooperage, confirming its high potential in this regard.

KEYWORDS: Oak aging, *Q. pyrenaica*, *Q. alba*, *Q. petraea*, volatile substances, ellagitannins

INTRODUCTION

Traditionally, great red wines are aged in oak barrels, because during this process the wine gains in complexity and stability (Garde-Cerdán and Ancín-Azpilicueta, 2006; Zamora, 2019). The oak wood enriches the wine with volatile substances that contribute to improving its quality by enhancing its aroma and flavour (Navarro *et al.*, 2018), as well as with phenolic compounds that contribute to the textural sensations it acquires (Navarro *et al.*, 2016a). All these compounds are either naturally present in the original oak wood or are produced during the toasting process of the barrels (Chira and Teissedre, 2013).

Oak wood can release several volatile substances, but only some of them will have a significant impact on wine aroma (Cadaña *et al.*, 2003). The main volatile substances released by oak wood to wine are furans, phenolic aldehydes and ketones, volatile phenols and β -methyl- γ -octalactones (Garde-Cerdán *et al.*, 2004; Prida and Chatonnet, 2010).

Furans are produced from wood polysaccharides due to the Maillard reaction which occurs during the toasting process needed to shape the barrel, as well as to mellow the wood's harsh ellagitannins, to mitigate raw oak flavours and to generate several new aromas to increase the intensity and complexity of wine (Boidron *et al.*, 1988; Fors, 1998). It is generally accepted that the family of furans (mainly furfural, methylfurfural, hydroxymethylfurfural and furfuryl alcohol) provide hints of smoky and toasted nuts (Spillman *et al.*, 2004), but they are usually below the perception threshold (Navarro *et al.*, 2018), and therefore their contribution to wine aroma is not significant. However, furfural can react with hydrogen sulfide to form furfurylthiol, an interesting volatile substance with a very pleasant coffee aroma (Tominaga *et al.*, 2000), and thus its impact cannot be ignored.

Phenolic aldehydes and ketones are produced from the thermal degradation of lignin during barrel toasting (Fors, 1998; Cadaña *et al.*, 2003). This family of compounds is responsible for the much-appreciated vanilla aroma of aged wines (Spillman *et al.*, 2004). Vanillin is the main contributor, but other compounds of this family, such as syringaldehyde, acetovanillone and propiovanillone, also contribute to this aroma (Prida and Chatonnet, 2010).

A heterogeneous family of compounds grouped under the name of volatile phenols includes eugenol, trans-isoeugenol, guaiacol, methylguaiacol, ethylguaiacol, vinylguaiacol, ethylphenol and vinylphenol as main substances (Garde-Cerdán and Ancín-Azpilicueta, 2006; Zamora, 2019). While some of these volatile phenols have a positive effect, others can play a positive or negative role depending on the concentration level. In addition, there are others which are clearly detrimental to the wine's aromatic quality. Eugenol and related substances enrich the wine with a very elegant clove aroma (Boidron *et al.*, 1988). Other volatile phenols, such as guaiacol and related compounds, contribute smoked and toasted notes (Chatonnet *et al.*, 1999) and, depending

on their relative concentration, can be positive or negative for wine quality: the effect is pleasant when their presence provides slight notes of toasted bread, but unpleasant when their concentrations are so high that burnt bread notes can be perceived (Navarro *et al.*, 2018). Finally, ethylphenol and vinylphenol cause very disagreeable odours of horse sweat and medicinal notes respectively. However, these compounds are mainly produced by spoilage microorganisms (essentially by *Brettanomyces*) resulting from the decarboxylation of coumaric acids (Chatonnet *et al.*, 1992).

β -methyl- γ -octalactones are responsible for coconut flavour in wine. These compounds, also known as whiskey lactones, are present in the form of two isomers (*cis* and *trans*). The perception threshold of the *cis* isomer is much lower than that of the *trans* isomer. Consequently, the contribution of *cis* isomer to coconut perception is much more significant than that of *trans* isomer (Abbott *et al.*, 1995).

Oak wood also releases phenolic compounds into the wine, particularly ellagitannins (Zhang *et al.*, 2015), which contribute to the mouthfeel and texture of wine, especially astringency and structure (Glabasnia and Hofmann, 2006; Michel *et al.*, 2011). The major ellagitannins from oak wood are castalagin, vescalagin, grandinin and roburins (Jourdes *et al.*, 2009).

All these compounds are released into the wine by oak wood, but the proportion will vary depending on different factors related to the barrel, such as the botanical and geographical origin of the tree its wood came from, seasoning time, degree of toasting and the number of times it has been previously used (Navarro *et al.*, 2016; Navarro *et al.*, 2018).

The *Quercus* genus includes more than 500 species of tree, but only three of them currently monopolise cooperage use (Chatonnet and Dubourdieu, 1998, Vivas, 2002, Zamora, 2019): the pedunculate oak (*Quercus robur*) and the sessile oak (*Quercus petraea*), which are commonly known as French oaks, and the American white oak (*Quercus alba*). It is widely known that *Q. petraea* and *Q. alba* provide greater aromatic richness than *Q. robur*; whereas *Q. robur* releases more ellagitannins (Masson *et al.*, 1995, Feuillat *et al.*, 1998, Ribéreau-Gayon *et al.*, 2006b, Navarro *et al.*, 2016, Navarro *et al.*, 2018). Since consumers nowadays prefer wines with aromatic complexity rather than with high structure, *Q. petraea* and *Q. alba* are the main oaks used for wine aging (Zamora, 2019). Other oak species have also been used in cooperage, but to a much lesser degree (Mosedale *et al.*, 1999).

Moreover, aging wine in oak barrels also leads to natural microoxygenation through wood porosity, the bunghole and the interstices between the staves (del Alamo-Sanza and Nevares, 2018). This oxygen supply leads to the formation of ethanal from ethanol that can react with flavan-3-ols to form a highly reactive carbocation, which quickly reacts to produce ethyl-bridged flavanol-flavanol adducts and/or flavanol-anthocyanin oligomers (Escribano-Bailón *et al.*, 2001). Ethanal can also contribute to the formation of vitisin B and other pyranoanthocyanins (Mateus *et al.*, 2002).

It is generally accepted that these reactions are linked to colour stabilisation and astringency softening in red wines (Ribéreau-Gayon *et al.*, 2006b).

Several studies have reported how barrels made of French oak (*Q. petraea* and *Q. robur*) and American oak (*Q. alba*) can contribute to wine composition and quality (Boidron *et al.*, 1988; Chatonnet and Dubourdieu, 1998; Cadahía *et al.*, 2003; Garde-Cerdán and Ancin-Azpilicueta, 2006; Prida and Chatonnet, 2010; Michel *et al.*, 2011; Chira and Teissedre, 2013; Chira and Teissedre, 2015; Navarro *et al.*, 2016; Navarro *et al.*, 2018; González-Centeno *et al.*, 2021); however, little is known about barrels made from other tree species not belonging to the *Quercus* genus (Young *et al.*, 2010; Fernández de Simón *et al.*, 2009; Sanz *et al.*, 2011), or even about barrels made from other *Quercus* species (Fernández de Simón *et al.*, 2008; YanLong *et al.*, 2010).

Of all the possible alternatives to classic cooperage oaks within the *Quercus* genus, *Q. pyrenaica* (commonly known as *Rebollo*) stands out because it is widespread (covering approximately 762,000 ha), especially throughout the Iberian Peninsula (Fernández-Parajes *et al.*, 2005), and because previous studies have shown it to have great potential (Cadahía *et al.*, 2001; Jordao *et al.*, 2005; Cadahía *et al.*, 2007; Castro-Vázquez *et al.*, 2013; Martínez-Gil *et al.*, 2020). However, to our knowledge, there is only one previous scientific article that has examined *Q. pyrenaica* barrels and wine (Fernández de Simón *et al.*, 2008). The aim of this research was therefore to examine how *Q. pyrenaica* barrels affect wine composition and wine quality in comparison with barrels of the most commonly used oaks, *Q. petraea* and *Q. alba*. This study was performed over two vintages in order to compare new barrels with barrels that have been used for one year (referred to hereafter as ‘one-year-used’).

MATERIALS AND METHODS

1. Chemicals

Methanol, acetonitrile, formic acid, acetic acid, ethyl acetate and absolute ethanol were of HPLC grade and purchased from Panreac (Barcelona, Spain). Acetaldehyde, phloroglucinol, ascorbic acid and sodium acetate were purchased from Sigma-Aldrich (Madrid, Spain). Dichloromethane (LiChrosolv quality) was purchased from Merck (Darmstadt, Germany) and pentane from Fluka (Buchs, Switzerland). The standards for proanthocyanidin analysis were: (+)-Catechin (≥ 99 %), (–)-epicatechin (≥ 99 %), (–)-epigallocatechin (≥ 98 %), and (–)-epicatechin-3-O-gallate (≥ 97.5 %). These were purchased from Extrasynthese (Genay, France). Standards for ellagitannin analysis: castalagin (99 %), vescalagin (98 %), roburin A (98 %), roburin B (93 %), roburin C (93 %), roburin D (98.5 %), grandinin (98.5 %) and roburin E (97 %). These ellagitannins were provided by the Institut des Sciences Moléculaires (ISM, Talence, France) using the services of ADERA (Talence, France). The chemical standards for gas chromatography were purchased from Aldrich and PolyScience (Niles, USA). The rest of the chemicals were of high purity and purchased from Panreac

(Barcelona, Spain). Pure water was obtained from a Milli-Q purification system (Millipore, USA).

2. Barrels

A total of 6 new barrels (225 L) were purchased from the Boteria Torner Cooperage (Sant Cugat Sesgarrigues, Barcelona, Spain). The cooperage selected the wood staves according to the criteria that they should be as homogeneous as possible within each species. Two barrels were made of American white oak (*Quercus alba*), two of French oak (*Quercus petraea*) and two of Iberian oak (*Quercus pyrenaica*). All the barrels were medium toasted.

3. Experimental design

This study was carried out with Cabernet-Sauvignon wines from two consecutive vintages, 2016 and 2017, from the Tarragona AOC (Spain), after malolactic fermentation had completely finished. The chemical characteristics of these red wines were very similar. Specifically, these were (for 2016 and 2017 vintages respectively): ethanol content: 13.4 % and 13.7 % (v/v); titratable acidity: 5.6 g/L and 5.2 g/L (expressed as tartaric acid); volatile acidity: 0.37 g/L and 0.45 g/L (expressed as acetic acid); pH: 3.61 and 3.76; total phenolic index: 52.3 and 53.5; free sulfur dioxide: 26 mg/L and 28 mg/L and total sulfur dioxide: 52 mg/L and 60 mg/L. The wine of the 2016 vintage was put into the new barrels in April 2017 and left to age for 12 months. Once the barrels were emptied and cleaned, they were immediately filled with wine from the 2017 vintage, which was also aged for 12 months. In this way, it was possible to study the effect on wine of new barrels made from three different species, as well as that of one-year-used barrels. The wines were sulfited when necessary in order to maintain the free sulfur dioxide levels between 25 mg/L and 30 mg/L. After twelve months of aging, the wines from both vintages were bottled and stored at 12 °C. Analyses were done immediately after bottling. A sensory analysis was carried out two months after bottling the wines from the second vintage (2017).

4. General analysis

Analytical methods recommended by OIV (OIV, 2021) were used to determine ethanol content, titratable acidity, volatile acidity, free SO₂ and total SO₂.

5. Spectrophotometric analysis

Absorbance measurements were performed using a Helios Alpha UV–vis spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Colour intensity (CI), total anthocyanins (TA) and *PVPP Index* were estimated following the methods described by Glories (1984). The CIELab coordinates – lightness (*L**), chroma (*C**), hue (*h**), red-greenness (*a**), and yellow-blueness (*b**) – were determined according to Ayala *et al.* (1997) and data processing was performed with MSCV software (Ayala *et al.*, 2001).

6. Anthocyanins, tannins and related parameters

The total phenolic index (TPI) was determined by measuring absorbance at 280 nm, and expressed as absorbance units (Ribéreau-Gayon *et al.*, 2006a).

Total anthocyanins (TA) and the *PVPP Index* were estimated following the methods described by Glories (1984). Total tannin concentration was estimated by precipitation with methylcellulose (Sarneckis *et al.*, 2006). Acid-catalysed depolymerisation of proanthocyanidins in the presence of an excess of phloroglucinol (phloroglucinolysis method) was used to analyse the proanthocyanidin content, monomeric composition and mean degree of polymerisation (mDP) of all the wines according to the procedure reported by Kennedy and Jones (2001).

7. Volatile compound analysis

The volatile compounds released from the oak wood were analysed by GC/MS using a Focus-GC system gas chromatograph coupled to a ISQ mass spectrometer with an electron impact ionisation source and quadrupole analyser equipped with a TriPlus autosampler, all from ThermoQuest. The conditions of the detector were as follows: electron multiplier voltage of 1250 V, impact energy of 70 eV, ion source temperature of 250 °C and mass scanning range of 40–250 amu. A BP21 column (SGE) with an internal diameter of 60 m – 0.32 mm and 0.25 mm thick free fatty acid phase (FFAP) (polyethylene glycol treated with nitroterephthalic acid) was used.

The volatile compounds were extracted and analysed using the method developed by Ibarz *et al.* (2006). 25 mL of wine was passed through columns filled with 0.2 g of LiChrolut EN (40–120 µm, Merck) using 4-nonanol as the internal standard. The columns were then washed with 25 mL of water to remove sugars, acids and other polar substances. The fraction of volatile compounds was eluted with 15 mL of pentane: dichloromethane (2:1 v/v). The extracts were concentrated by distillation in a Vigreux column and then under nitrogen stream to 100 µL. They were then kept at -20 °C until analysis.

The separated compounds were identified by their mass spectra and chromatographic retention times, using commercial products as a standard. Quantification was performed by analysing the characteristic m/z fragment for each compound using the internal standard method. The results for the non-available compounds were expressed in concentration units (µg/L or mg/L) as internal standard equivalents, which were obtained by normalising the compound peak to that of the internal standard and multiplying them by the concentration of the internal standard. Only the main volatile compounds released by oak wood (furanic compounds, vanillin, β-methyl-γ-octalactones and volatile phenols) are shown here, because the aim of this study was to focus on the aromas released by wood.

8. Ellagitannin analysis

The ellagitannins were analysed by HPLC using a modification of a previously described method (García-Estevéz *et al.*, 2010), which was adapted to use fused core C18 chromatographic columns (Ascentis Express, 150 × 4.6 mm, 2.7 µm particle size), thermostatted at 40 °C (Navarro *et al.*, 2017). The ellagitannins were identified by matching the retention time and spectral data

(DAD-UV-vis and MS/MS) against those of authentic standards. The quantitation was performed using the DAD-chromatograms extracted at 250 nm.

9. Sensory analysis

All sensory analyses were performed in the tasting room of the Faculty of Oenology of Tarragona (Universitat Rovira i Virgili), which was designed in accordance with UNE 87004.197 (AENOR, 2010). Tasting was carried out using ISO official tasting glasses (ISO-3591, 1997). Each sample consisted of 30 mL of wine at room temperature (20 °C), covered with clear plastic petri dishes to minimise the escape of volatile components and randomly coded with three-digit numbers.

All the samples were tasted by 20 students in the final year of the Bachelor's Degree in Oenology at the Universitat Rovira i Virgili (a four-year degree). This panel was made up of 12 men and 8 women aged between 21 and 25, who had been training together for 3 years while studying sensory analysis as part of their oenology degree. To simplify the tasting, the wines from two similar (duplicate) samples of each experimental group were mixed.

Two months after the bottling of the second vintage (2017), triangular tests and sensory descriptive analyses were carried out; these were spread over two sessions to prevent fatigue in the panelists. During the first session, 6 trials of sensory triangle tests were conducted in accordance with ISO-4120 (2004). The triangular tests performed were: 1) new French barrels vs. new American barrels, 2) new French barrels vs. new *Q. pyreanica* barrels, 3) new American barrels vs. new *Q. pyreanica* barrels, 4) one-year-used French barrels vs. one-year-used American barrels, 5) one-year-used French barrels vs. one-year-used *Q. pyreanica* barrels, and 6) one-year-used American barrels vs. one-year-used *Q. pyreanica* barrels. The second session was a descriptive sensory analysis performed in accordance with ISO-13299 (2003). During this session, the tasters were required to evaluate the intensity of three olfactory sensory attributes (Coconut, Vanilla and Smoked/Toasted notes) and two mouthfeel sensory attributes (Astringency and Structure) on a scale of 1 to 10 (1 = slight intensity and 10 = maximum intensity). The intensity level of each descriptor was then expressed as the mean value of all the results. A sensory training session was held beforehand so that the panelists could agree on the criteria for each of the different sensory attributes. Since, in wineries, barrels are usually selected on the basis of the winemakers' criteria, the tasters were also asked which of the samples they preferred. The aim of this question was not to determine the preferences of the consumers, but to discover which type of barrel would be selected by the trained professionals responsible for such decisions. Samples were served randomly to avoid any influence arising from the tasting order.

10. Statistics

All analytical data are expressed as the arithmetic average ± standard deviation of three replicates. All sensory data are expressed as the arithmetic mean ± standard deviation of

the scores of the 20 tasters. One-factor analysis of variance (ANOVA) was carried out using SPSS 15.0 software (SPSS Inc., Chicago, IL). The level of significance of the sensory triangle tests was determined following the method described by Jackson (Jackson, 2002).

RESULTS AND DISCUSSION

1. Wine colour

Table 1 shows a comparison of the colour intensity and the CIEL*a*b* coordinates of wines of both vintages after twelve months of aging in the different oak barrels with the corresponding original wines at the beginning of the experiment.

In general, the colour intensity (CI), chroma (C*) and green-red component (a*) of all the barrel-aged wines tended to

decrease in comparison with the original wine, whereas lightness (L*), hue (h*) and the blue-yellow component (b*) did the opposite. This behaviour was observed in both vintages and consequently in the new and one-year-used barrels. These data show that wine colour followed its natural evolution in both vintages and in all barrel types, since the intensity of the red component diminished and the yellowish nuances increased (Ribéreau-Gayon *et al.*, 2006b; Oberholster *et al.*, 2015). However, no significant differences were found in the wines of either vintage in relation to the type of barrel in which they were aged. This data suggests that the oxygen transfer rate across the wooden barrels was similar in the different oak tree species, since oxygen is probably the main factor responsible for the colour changes during wine aging (Ribéreau-Gayon *et al.*, 2006b; Zamora, 2019; Sánchez-Gómez *et al.*, 2020).

TABLE 1. Colour parameters.

Parameter	Vintage	Barrel age	Original wine	<i>Q. petraea</i>	<i>Q. alba</i>	<i>Q. pyrenaica</i>
CI	2016	New	13.3 ± 0.3 B	12.4 ± 0.2 A	12.6 ± 0.3 A	12.7 ± 0.4 A
	2017	1 year	14.8 ± 0.1 B	13.6 ± 0.3 A	13.4 ± 0.4 A	13.8 ± 0.3 A
L*	2016	New	45.2 ± 0.6 A	48.3 ± 0.9 B	47.9 ± 0.6 B	48.4 ± 0.9 B
	2017	1 year	41.2 ± 0.6 A	44.3 ± 0.9 B	44.2 ± 1.5 B	45.8 ± 0.3 B
C*	2016	New	58.1 ± 0.7 B	56.2 ± 0.9 A	56.0 ± 0.5 A	56.4 ± 0.2 A
	2017	1 year	60.1 ± 0.4 B	58.5 ± 0.4 A	58.7 ± 0.3 A	59.2 ± 0.3 A
h*	2016	New	15.1 ± 0.7 A	17.8 ± 1.0 B	18.1 ± 1.3 B	19.1 ± 1.9 B
	2017	1 year	20.2 ± 0.6 A	22.3 ± 0.4 B	22.4 ± 1.1 B	21.8 ± 0.4 B
a*	2016	New	53.6 ± 0.3 B	51.2 ± 0.4 A	52.0 ± 0.7 A	51.7 ± 0.6 A
	2017	1 year	53.6 ± 0.3 B	51.2 ± 0.4 A	52.0 ± 0.7 A	51.7 ± 0.6 A
b*	2016	New	15.0 ± 0.5 A	16.7 ± 0.9 B	16.8 ± 0.4 B	17.1 ± 0.2 B
	2017	1 year	17.6 ± 0.3 A	19.3 ± 1.1 B	19.6 ± 0.9 B	20.1 ± 0.3 B

All data are expressed as the average values of 2 replicates ± standard deviation. Different Capital letters indicate the existence of statistical differences ($p < 0.05$). CI: colour intensity; L*: lightness value (CIElab coordinates); C*: chroma value (CIElab coordinates); h*: hue value (CIElab coordinates); a*: green-red component (CIElab coordinates); b*: blue-yellow component (CIElab coordinates).

2. Anthocyanins, tannins and related parameters

Table 2 shows the anthocyanin and PVPP index of the wines aged in the different oak barrels in comparison with the corresponding original wines.

As expected, the total anthocyanin concentration decreased over time in all the experimental conditions, which confirmed that oxygen can degrade anthocyanins, as has been previously reported (King *et al.*, 1980). However, the PVPP index tended to increase over time in all the wines, regardless of the oak species used for the oak barrel or whether the barrels were new or used for one year. Since the PVPP index indicates the percentage of anthocyanins combined with flavanols (Glories, 1984), these results confirm that oak

aging favours the union of anthocyanins and flavanols via the formation of ethyl bridges (Ribéreau-Gayon *et al.*, 1983; Atanasova *et al.*, 2002; Llaudy *et al.*, 2006). Because these new pigments are more stable than free anthocyanins, their formation has been associated with the stabilisation of the red wine colour (Ribéreau-Gayon *et al.*, 2006b; Fernandes *et al.*, 2017).

Table 2 also shows the tannin concentration and some related parameters of the wines aged in the different oak barrels in comparison with the corresponding original wines. The total phenolic index (TPI) of the 2016 vintage wine aged in the *Q. petraea* and *Q. Pyrenaica* barrels was significantly higher than the corresponding original wine, whereas no differences were found in the same wine aged in the *Q. alba* barrels.

TABLE 2. Anthocyanins, tannins and related parameters.

Parameter	Vintage	Barrel age	Original wine		<i>Q. petraea</i>		<i>Q. alba</i>		<i>Q. pyrenaica</i>	
TA (mg/L)	2016	New	431 ± 17	B	301 ± 17	A	309 ± 12	A	307 ± 22	A
	2017	1 year	457 ± 14	B	335 ± 31	A	328 ± 27	A	322 ± 17	A
PVPP Index (%)	2016	New	27.7 ± 2.4	A	38.2 ± 1.9	B	40.0 ± 4.1	B	41.3 ± 2.9	B
	2017	1 year	31.5 ± 2.1	A	41.3 ± 2.7	B	39.7 ± 2.5	B	42.3 ± 3.4	B
TPI	2016	New	52.3 ± 0.3	A	53.6 ± 0.5	B	51.7 ± 0.8	A	54.5 ± 0.4	B
	2017	1 year	53.5 ± 0.2	A	53.0 ± 0.8	A	52.6 ± 0.3	A	53.3 ± 0.4	A
Tannins (g/L)	2016	New	1.67 ± 0.12	A	1.45 ± 0.17	A	1.51 ± 0.18	A	1.48 ± 0.14	A
	2017	1 year	1.82 ± 0.19	A	1.74 ± 0.17	A	1.72 ± 0.21	A	1.69 ± 0.12	A
PA (mg/L)	2016	New	618 ± 53	B	512 ± 45	A	487 ± 67	A	503 ± 56	A
	2017	1 year	674 ± 59	B	568 ± 37	A	554 ± 41	A	549 ± 59	A
mDP	2016	New	4.78 ± 0.37	A	4.85 ± 0.31	A	4.63 ± 0.24	A	4.92 ± 0.19	A
	2017	1 year	4.83 ± 0.26	A	5.12 ± 0.27	A	5.00 ± 0.18	A	5.08 ± 0.39	A
% PD	2016	New	15.8 ± 0.4	A	16.0 ± 0.3	A	15.3 ± 0.5	A	15.0 ± 0.5	A
	2017	1 year	16.7 ± 0.3	A	16.7 ± 0.3	A	16.3 ± 0.7	A	16.0 ± 0.8	A
% GAL	2016	New	5.10 ± 0.15	A	5.20 ± 0.15	A	5.31 ± 0.19	A	5.12 ± 0.25	A
	2017	1 year	4.98 ± 0.12	A	5.00 ± 0.35	A	4.97 ± 0.22	A	5.07 ± 0.13	A

All data are expressed as the average values of 2 replicates ± standard deviation. Different Capital letters indicate the existence of statistical differences ($p < 0.05$). TA: Total anthocyanins; PVPP Index (%): the percentage of anthocyanins combined with flavanols; TPI: total phenolic index; PA: Proanthocyanidins; mDP: Mean degree of polymerisation; % PD: Percentage of prodelfhinidins; % GAL: Percentage of galloylation.

These results may be due to the fact that *Q. petraea* and *Q. pyrenaica* are richer in ellagitannins and other phenolic compounds than *Q. alba* (Chatonnet and Dubourdiu, 1998; Cadahía *et al.*, 2001; Castro-Vázquez *et al.*, 2013; Navarro *et al.*, 2016). By contrast, no differences were found in the TPI in the case of the 2017 vintage, regardless of the oak barrel type, probably because these wines were not aged in new barrels but in one-year-old barrels. The reduction in the capacity of one-year-used barrels to release ellagitannins and other phenolic compounds may explain these results. (Navarro *et al.*, 2016; Zamora, 2019).

The tannin concentration measured by the methyl cellulose precipitation method (Sarneckis *et al.*, 2006) tended to decrease in all the wines aged in oak barrels, but the differences were not significant. This trend was also observed in the concentration of proanthocyanidins quantified by phloroglucinolysis (Kennedy and Jones, 2001); in this case, however, the differences were significant. This decrease could be due to a precipitation of the large polymers during aging (Waters *et al.*, 1994; Smith *et al.*, 2015), as well as to a lower effectiveness of phloroglucinol in cleaving down the increasingly complex chemical structures of proanthocyanidins (Foo *et al.*, 2000; Zeng, 2015). In any case, no differences were found in either of the two vintages aged in the different oak barrels in terms of their concentration of tannins or proanthocyanidins, their mean degree of polymerisation (mDP), their percentage of prodelfhinidins (%PD), or their percentage of galloylation (%GAL).

3. Volatile substances released by oak wood

Table 3 shows the concentrations of the main volatile substances released by the oak wood into the different wines. The various volatile substances released from the different oak barrels were grouped into four families (furanic compounds, phenolic aldehydes and ketones, β -methyl- γ -octalactones and volatile phenols) in order to facilitate the discussion of the results. Due to their high sensory importance, the following substances have also been shown individually: furfural within the furans, vanillin within the phenolic aldehydes and ketones, the *cis* and *trans* isomers of the β -methyl- γ -octalactone, as well as the percentage of the *cis* isomer, and eugenol within the volatile phenols. The complete data about furans, the phenolic aldehydes and ketones, and volatile phenols are shown in Supplementary Tables S1-S3 respectively. Data of the concentration of these compounds in the original wines are not provided as their concentration was very low, given that they had not been in contact with wood before the beginning of the experiment.

The highest levels of furans were found in the wines of the 2016 vintage aged in new *Q. petraea* barrels, followed in descending order by the wines aged in *Q. pyrenaica* and *Q. alba*. This behaviour was also observed for furfural. As expected, the concentration of total furans, and in particular of furfural, was significantly lower in all the 2017 vintage wines, since they were aged in one-year-used barrels. Surprisingly, all the wines of this vintage had similar values of total furans and furfural, regardless of the species of oak. In any case,

TABLE 3. Main volatile substances released by wood.

Compound (µg/L)	<i>Q. petraea</i>		<i>Q. alba</i>		<i>Q. pyrenaica</i>	
	New	1 year	New	1 year	New	1 year
Total furans	2057 ± 250 C β	415 ± 141 A α	1024 ± 231 A β	501 ± 136 A α	1493 ± 174 B β	504 ± 101 A α
Furfural	514 ± 140 B β	158 ± 54 A α	217 ± 37 A α	146 ± 80 A α	469 ± 26 B β	209 ± 66 A α
Total phenolic aldehydes and ketones	3360 ± 38 B β	1114 ± 38 A α	2550 ± 267 A β	1772 ± 395 B α	2370 ± 737 A β	943 ± 194 A α
Vanillin	499 ± 21 A β	195 ± 9 B α	428 ± 42 A β	285 ± 46 C α	362 ± 88 A β	133 ± 16 A α
total BMGO	186 ± 9 B β	111 ± 23 A α	677 ± 166 A β	416 ± 81 B α	604 ± 47 A β	166 ± 48 B α
<i>t</i> -BMGO	40 ± 6 B β	23 ± 3 A α	56 ± 21 A α	33 ± 6 A α	165 ± 10 B β	61 ± 22 A α
<i>c</i> -BMGO	146 ± 3 A β	88 ± 19 A α	621 ± 145 A β	383 ± 75 A α	439 ± 37 A β	105 ± 25 A α
% <i>cis</i> isomer	78 ± 3 A α	79 ± 1 A α	92 ± 1 A α	92 ± 1 A α	73 ± 6 A α	63 ± 5 A α
Total Volatile Phenols	1989 ± 280 B α	1960 ± 424 B α	2102 ± 298 B α	1758 ± 498 B α	2541 ± 386 B α	2032 ± 508 B α
Eugenol	92 ± 16 B β	50 ± 11 B α	30 ± 8 A β	11 ± 1 A α	100 ± 4 B β	38 ± 7 B α

All data are expressed as the average values of 2 replicates ± standard deviation. Different capital letters indicate the existence of statistical differences ($p < 0.05$) between wines aged in barrels of different oak species and same time of use. Different Greek letters indicate the existence of statistical differences ($p < 0.05$) between wines aged in barrels of the same oak species and different time of use. Total BMGO: Total β-Methyl-γ-octalactones; *t*-BMGO: *trans*-β-Methyl-γ-octalactone; *c*-BMGO: *cis*-β-Methyl-γ-octalactone.

this family of compounds is formed from carbohydrates and nitrogen compounds by means of the Maillard reaction, and consequently their final concentration in wine is more closely related to the toasting level of the barrels than to the genetic origin of the oak (Chatonnet *et al.*, 1999; Cadahía *et al.*, 2003; Navarro *et al.*, 2018). In addition, the levels of furans of these wines is below the perception threshold, and therefore their impact on the wine aroma does not appear to be of great importance (Navarro *et al.*, 2018).

The maximal concentration of phenolic aldehydes and ketones was found in the 2016 vintage wine aged in *Q. petraea* barrels, whereas it was about 25 % lower in the corresponding wines aged in *Q. alba* and *Q. pyrenaica* barrels. A similar trend was observed for vanillin. Once again the levels of this family of volatile substances, including vanillin, were much lower in the 2017 vintage wine, since they were aged in one-year-used barrels. As in the case of furans, phenolic aldehydes and ketones are produced as a result of the thermal degradation of lignin during barrel toasting (Fors, 1998; Cadahía *et al.*, 2003), and therefore their final concentration in the wine depends more on the level of barrel toasting than on the genetic origin of the oak.

As expected, the 2016 vintage wine aged in new *Q. alba* barrels had significantly higher total concentrations of β-methyl-γ-octalactona (BMGO) than those aged in new *Q. petraea* barrels. Moreover, the percentage of *cis*-BMGO, the isomer with the lowest perception threshold (Abbott *et al.*, 1995), was also much higher in the wines aged in the new *Q. alba* barrels (around 92 %) than in the new *Q. petraea* barrels (around 72 %). This data agrees with previously published information (Chatonnet and Dubourdiou, 1998;

Cadahía *et al.*, 2003; Navarro *et al.*, 2018) and confirms that wines aged in American oak barrels contribute to a much greater sensation of coconut notes than wines aged in French oak. In contrast, wines of the same vintage aged in the new *Q. pyrenaica* barrels contained a similar total concentration of BMGO than those aged in the new *Q. alba* barrels, but they contained a proportion of *cis*-isomer (73 %) similar to the wines aged in the new *Q. petraea* barrels. Consequently, the wines aged in the new *Q. pyrenaica* barrels had a much higher sensory impact in terms of coconut notes than those aged in the new French oak barrels, but they were not as high as those aged in the new American oak barrels. As expected, the wines of the 2017 vintage aged in the one-year-used barrels generally had a lower total BMGO content than the wines of the preceding vintage that were aged in the new barrels. However, it must be highlighted that the levels of BMGO in the wines aged in the one-year-used *Q. pyrenaica* barrels were much lower than those of the wines aged in the one-year-used *Q. alba* barrels and similar to those of the wines aged in one-year-used *Q. petraea* barrels. It seems therefore that the depletion of BMGO is faster in *Q. pyrenaica* barrels than in *Q. alba* barrels. All these data are important, because the levels of BMGO depend on the toasting level of the barrels and on the genetic origin of the oak tree, especially the percentage of the *cis*-isomer.

Similar total concentration values for volatile phenols were found in all the wines regardless of the vintage, the oak species or whether the barrel was new or one-year-used. These results are to be expected, because volatile phenols, in addition to being produced from lignin during barrel toasting (Chira and Teissedre, 2013; Zamora, 2019), can also be produced during aging as a result of the microbiological

decarboxylation of phenol acids (Chatonnet *et al.*, 1992). Even more significant is the influence of the oak species on the levels of eugenol, a very pleasant substance with an elegant clove aroma. Wines of the 2016 vintage aged in the new *Q. petraea* and *Q. pyrenaica* barrels showed maximal levels of eugenol, which were around three times higher than in similar wines aged in the new *Q. alba* barrels. As expected, the eugenol concentration was much lower in the 2017 vintage wines aged in the one-year-used barrels. However, wines aged in the one-year-used *Q. petraea* and *Q. pyrenaica* barrels maintained higher concentrations of eugenol than those aged in the corresponding *Q. alba* barrels.

4. Ellagitannins

Table 4 shows the ellagitannin content of the wines aged in the different oak barrels. The 2017 vintage wines aged in the new *Q. petraea* and *Q. pyrenaica* barrels showed much higher concentrations of ellagitannins - around 2.7 times higher than those aged in new *Q. alba* barrels. This behaviour was similar for all ellagitannins, castalagin being the most abundant in all cases. This difference in the levels of ellagitannins between *Q. petraea* and *Q. alba* is well-known (Masson *et al.*, 1995; Chatonnet and Dubourdieu, 1998; Cadahía *et al.*, 2001; Navarro *et al.*, 2016), but *Q. pyrenaica*'s high ellagitannin-releasing potential, very similar to that of *Q. petraea*, was not so evident. While it is true that some studies have reported the high richness of ellagitannins in *Q. pyrenaica*, all of those studies were performed using staves or other oak alternatives (Cadahía *et al.*, 2001; Jordao *et al.*, 2005; Cadahía *et al.*, 2007; Castro-Vázquez *et al.*, 2013; Martínez-Gil *et al.*, 2020) as opposed to a complete barrel aging process.

As expected, the 2017 vintage wines that were aged in one-year-used barrels showed an ellagitannin concentration significantly lower than corresponding wines of the 2016 vintage that were aged in new barrels. However, the wines aged in the one-year-used *Q. petraea* and *Q. pyrenaica*

barrels maintained higher levels of ellagitannins than those aged in *Q. alba*. These results confirm therefore that the ellagitannin-releasing potential of *Q. pyrenaica* is similar to that of *Q. petraea*.

5. Principal component analysis

A principal component analysis was performed to better understand the main differences related to these three oak species. Figure 1 shows the plot of a varimax-rotated principal component analysis of the different wines. This statistical analysis was performed with only three parameters: *cis*- β -methyl- γ -octalactone, eugenol and total ellagitannins. No further variables were needed to obtain a complete separation of the wines aged in the different oak barrels. The first component explains 65.23 % of the variance and the second component explains 34.10 %; the aggregate variance of these first two components was 99.33 %.

The PCA allowed us to separate the wines from the different barrel types with the exception of the one-year-used *Q. petraea* and *Q. pyrenaica* barrels, which were plotted very close to each other. PC1 placed the wines aged in the new *Q. petraea* and *Q. pyrenaica* barrels on the right, whereas wines aged in the new *Q. alba* barrels and all the wines aged in the one-year-used barrels are on the left. In contrast, PC2 placed the wines aged in the new and one-year-used *Q. alba* barrels and the wines aged in the new *Q. pyrenaica* barrels at the top of the graph, whereas the wines aged in the new and one-year-used *Q. petraea* barrels and the wine aged in the one-year-used *Q. pyrenaica* barrels are at the bottom.

The loadings are shown as arrows, the length and direction of which indicate the contribution made by the two components. The arrow corresponding to the total ellagitannins and eugenol points to the right, indicating that the samples placed further towards the right have a higher concentration of these compounds. By contrast, the arrow corresponding to *cis*- β -methyl- γ -octalactone points upwards, indicating that

TABLE 4. Ellagitannins.

Compound (mg/L)	<i>Q. petraea</i>		<i>Q. alba</i>		<i>Q. pyrenaica</i>	
	New	1 year	New	1 year	New	1 year
Vescalin	0.34 ± 0.12 B β	0.09 ± 0.01 B α	0.11 ± 0.03 A β	0.03 ± 0.02 A α	0.26 ± 0.09 B β	0.05 ± 0.02 A α
Castalin	1.65 ± 0.32 B β	0.54 ± 0.03 B α	0.60 ± 0.10 A β	0.25 ± 0.14 A α	1.78 ± 0.50 B β	0.37 ± 0.03 A α
Roburins A, B, C and D	1.32 ± 0.41 B β	0.26 ± 0.19 A α	0.48 ± 0.21 A β	0.13 ± 0.07 A α	1.30 ± 0.43 B β	0.08 ± 0.08 A α
Roburin E	2.62 ± 0.65 B β	0.89 ± 0.16 B α	0.99 ± 0.27 A β	0.45 ± 0.21 A α	3.57 ± 0.74 B β	0.98 ± 0.06 B α
Grandinin	2.10 ± 0.50 B β	0.61 ± 0.21 AB α	0.89 ± 0.22 A β	0.27 ± 0.14 A α	2.84 ± 0.40 B β	0.75 ± 0.08 B α
Vescalagin	1.49 ± 0.29 B β	0.52 ± 0.08 B α	0.57 ± 0.18 A β	0.18 ± 0.07 A α	1.08 ± 0.19 B β	0.32 ± 0.03 B α
Castalagin	7.98 ± 1.56 B β	3.29 ± 0.98 B α	3.13 ± 0.79 A β	1.42 ± 0.42 A α	8.46 ± 1.09 B β	2.56 ± 0.06 B α
Total Ellagitannins	17.50 ± 3.85 B β	6.20 ± 1.66 B α	6.78 ± 1.80 A β	2.73 ± 1.07 A α	19.28 ± 3.42 B β	5.11 ± 0.35 B α

All data are expressed as the average values of 2 replicates \pm standard deviation. Different capital letters indicate the existence of statistical differences ($p < 0.05$) between wines aged in barrels of different oak species and same time of use. Different Greek letters indicate the existence of statistical differences ($p < 0.05$) between wines aged in barrels of the same oak species and different time of use.

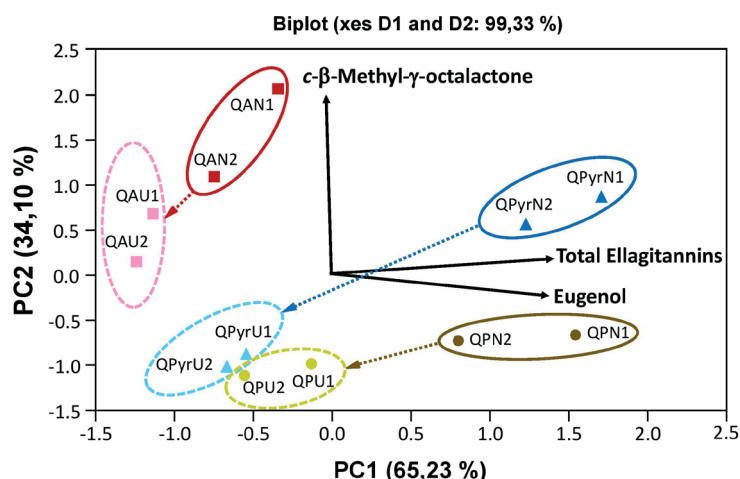


FIGURE 1. Plot of varimax-rotated principal component analysis.

QAN1 and QAN2: *Quercus alba* new barrels; QAU1 and QAU2: *Quercus alba* used barrels; QPN1 and QPN2: *Quercus petraea* new barrels; QPU1 and QPU2: *Quercus petraea* used barrels; QPyrN1 and QPyrN2: *Quercus pyrenaica* new barrels; QPyrU1 and QPyrU2: *Quercus pyrenaica* used barrels

samples placed further up are richer in this volatile substance. Another aspect to note is that all the wines aged in one-year-used barrels are found on the left and at the bottom in relation to the wines aged in their corresponding new barrels, which confirms the depletion of substances that oak wood can release over time.

This principal component analysis visually confirms that *Q. pyrenaica* has an ellagitannin and eugenol releasing capacity similar to that of *Q. petraea*, and of course much higher than that of *Q. alba*. Likewise, this PCA clearly shows that *Q. pyrenaica* wood has a *cis*-β-methyl-γ-octalactone releasing capacity much higher than that of *Q. petraea* when the barrels are new, and which is almost as high as that of *Q. alba*. However, after one year of aging, the *cis*-β-methyl-γ-octalactone releasing capacity of the *Q. pyrenaica* barrels decreased significantly until it reached levels similar to those of the (new or used) *Q. petraea* barrels.

6. Sensory analysis

Table 5 shows the results of the sensory comparison by triangle test between the wines aged in barrels made from the three different oak species. The comparison was performed by comparing only the three oak species aged in the new barrels in pairs and later in the one-year-used barrels.

The wines aged in the new barrels were not compared with the wines aged in the one-year-used barrels, because they were of a different vintage and consequently the differences would have been very large.

The panelists were able to significantly distinguish between the wines aged in the new barrels of the three species. However, they were not able to significantly distinguish between the wines aged in *Q. pyrenaica* and those aged in *Q. petraea* or *Q. alba* when the barrels had been used for one year. This means that the wine aged in the one-year-used *Q. pyrenaica* barrels was indistinguishable from the same wine aged in one-year-used *Q. petraea* and *Q. alba* barrels; this suggests that it has characteristics midway between these two types of oak after one year of use.

Finally, Figure 2 shows the results of the comparative descriptive sensory analysis of both the 2016 and 2017 vintage wines, which were aged in new and one-year-used barrels of the three oak species respectively. Evidently, the differences between wines of a different vintage may have had considerable influence on this analysis. However, the tasters were asked to primarily consider the sensory impact of the wood in order to minimise the effect of the vintage.

TABLE 5. Triangular test.

Triangular test		Positive indentifications	p value	Significance
New barrels	<i>Q. petraea</i> vs. <i>Q. alba</i>	14/20	0.00087	YES
	<i>Q. petraea</i> vs. <i>Q. pyrenaica</i>	11/20	0.0376	YES
	<i>Q. alba</i> vs. <i>Q. pyrenaica</i>	12/20	0.01297	YES
1 year used barrels	<i>Q. petraea</i> vs. <i>Q. alba</i>	11/20	0.0376	YES
	<i>Q. petraea</i> vs. <i>Q. pyrenaica</i>	9/20	0.19054	NO
	<i>Q. alba</i> vs. <i>Q. pyrenaica</i>	9/20	0.19054	NO

The differences were considered as significant when p value < 0.05

As expected, the panelists perceived a greater intensity in coconut aroma in the 2016 vintage wine aged in the new *Q. alba* barrels than in the same wine aged in the new *Q. petraea* barrels; however, they also perceived a high coconut intensity in the same wine aged in the new *Q. pyrenaica* barrels. This coconut perception diminished in the 2017 wines aged in the one-year-used barrels, but the intensity of this aroma was greater still in the wines aged in *Q. alba* and *Q. pyrenaica* than in the corresponding wine aged in *Q. petraea*. These results match very well with the concentrations of β -methyl- γ -octalactone found in these wines.

The highest intensity of vanilla aroma was perceived by the panelists in the 2016 vintage wine aged in the new *Q. petraea* barrels, followed in decreasing intensity by the same wine aged in the new *Q. alba* and in *Q. pyrenaica* barrels. Logically, the intensity of the perception of vanilla decreased in all wines of the following vintage, since they had been aged in the one-year-used barrels. However, the wines aged in *Q. petraea* and *Q. alba* maintained similar values to the 2016 vintage wine aged in the new *Q. pyrenaica* barrels. The 2017 vintage wine aged in the one-year-used *Q. pyrenaica* barrels showed the lowest intensity of vanilla aroma. These results also matched the vanilla concentration levels in the case of the wines aged in the new barrels. However, in the case of the wines aged in the one-year-used barrels, the parallel between the concentration of vanilla and the perception of vanilla aroma was not so clear.

In general, the panelists perceived similar intensities of smoked/toasted notes in the wines of the 2016 vintage aged in the new barrels regardless of the oak species, and the

intensity of these notes slightly decreased in wines of the 2017 vintage aged in the one-year-used barrels.

The perception of astringency and structure was quite similar across all the samples. However, the 2016 vintage wines aged in the *Q. alba* barrels showed slightly lower values for both attributes than their corresponding wines aged in the *Q. pyrenaica* and *Q. petraea* barrels. These data match very well with the total ellagitannin concentration of these wines, although it must be noted that astringency and structure also depend on many other factors, such as proanthocyanidin concentration and wine age. This trend was also observed in the 2017 vintage wines that were aged in the one-year-used barrels and correlates well with their ellagitannin concentration.

Finally, the panelists classified all 2016 vintage wines aged in the new barrels as better than the 2017 vintage wines aged in the one-year-used barrels, based on their personal preferences. This order of preference may be due to various factors, such as the intrinsic differences between the vintages, or the fact that the 2016 vintage underwent one more year of aging, which contributes to its development; however, it is also very likely that the greater presence of the oak's sensory attributes in wines aged in new barrels also exerts an important influence.

It is worth highlighting the order of preference always given by the panelists regardless of the vintage and whether the barrels were new or had been used for one year: first, wines aged in the *Q. petraea* barrels; second, wines aged in the *Q. pyrenaica* barrels; third, wines aged in the *Q. alba* barrels.

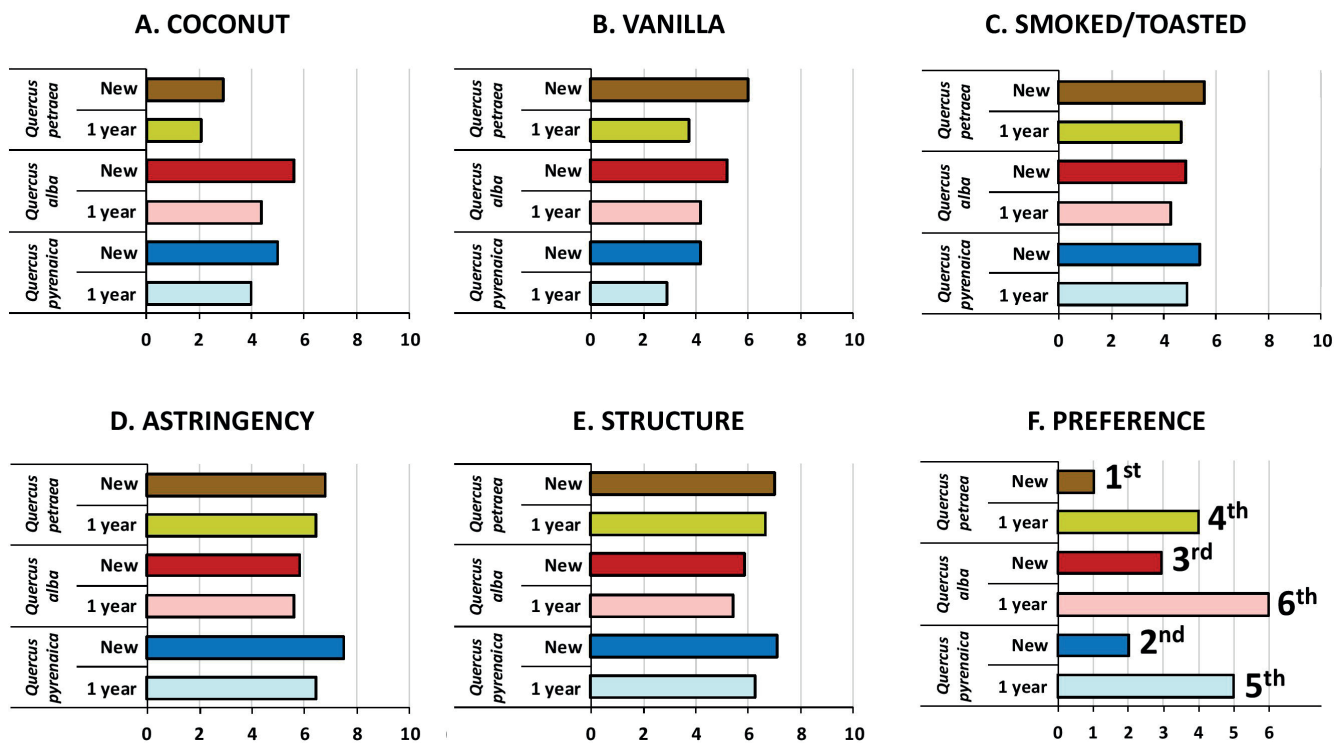


FIGURE 2. Descriptive sensory analysis.

CONCLUSIONS

This study confirms that the oak species *Quercus pyrenaica*, commonly called *Rebollo*, has great cooperage potential. The results show that wines aged in new *Quercus pyrenaica* barrels have similar levels of β -methyl- γ -octalactona to wines aged in new *Q. alba* barrels and clearly higher levels than wines aged in *Q. petraea*. Nevertheless, *Q. pyrenaica* releases lower levels of vanilla than *Q. alba* and particularly *Q. petraea*. These data indicate that *Q. pyrenaica* is more similar to American oak than French oak from an aromatic point of view, since it provides mainly coconut notes. By contrast, new *Q. pyrenaica* barrels release similar levels of ellagitannins to *Q. petraea* and clearly higher levels than *Q. alba*, indicating that it resembles French oak more than American oak in terms of wine structuration. Moreover, a trained panel ranked the wines aged in the *Q. pyrenaica* barrels in second position, just behind the wines aged in the *Q. petraea* barrels and ahead of those aged in *Q. alba*, based on their preferences. The *Q. pyrenaica* characteristics were sustained after one year of barrel use despite the natural depletion of substances. Consequently, *Q. pyrenaica* seems to have characteristics midway between the most commonly used oak species for cooperage, which confirms its great potential in this regard.

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