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# Evolution of red wine in oak barrels with different oxygen transmission rates. Phenolic compounds and colour

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ABSTRACT

Oxygen is an important factor for the quality of wine aged in barrels. The development of a tool to classify wood by its oxygen transmission rate (OTR) has made it possible to use the same batch of wood to make barrels that oxygenate twice as much as others. Therefore, the aim of this study was to evaluate the phenolic composition and colour of the same red wine aged for twelve months in barrels with a low oxygen transmission rate (LOTR) and with a high oxygen transmission rate (HOTR). The results showed that the same wine evolves differently in barrels with different oxygenation rates. Wines that received lower oxygenation (LOTR) had a higher polymeric pigment and total phenol content that were more polymerized. In these wines, the release of low molecular weight wood compounds was faster and higher than in the case of those aged in HOTR barrels. However, the wines from HOTR barrels maintained a higher level of anthocyanins and a more tannic character. Wines aged in LOTR barrels showed a higher colour intensity due to the increased formation of new pigments. These wines will maintain their red colour better than those aged in HOTR barrels.

## 1. Introduction

The barrel is an active vessel that releases compounds to the wine and allows oxygen to enter, which enhances the integration of these compounds with those in the wine. The amount of oxygen that is transferred to the wine during barrel ageing is known as the oxygen transmission rate (OTR) and plays a fundamental role in the ageing of red wine in barrels, as it determines the reactions that will occur during this process and, consequently, the final characteristics of the wine (del Alamo-Sanza & Nevares, 2017). Wine stored in barrels acquires complex aromas, stabilizes its colour and clarifies spontaneously improving the quality of the wine, this being indispensable in the production of high quality wines (Garde-Cerdán & Ancín-Azpilicueta, 2006; Li & Duan, 2019). Anthocyanins are the main compounds responsible for the purple-red colour of young wines. However, they are very unstable in their free form and participate in co-pigmentation reactions, polymerisation or the formation of new, more complex and stable pigments, which mainly arise from the interaction between anthocyanins and other phenolic compounds, during barrel ageing (Fernandes, Oliveira, Teixeira, Mateus, & de Freitas, 2017). These phenolic compounds include those from wood, such as ellagitannins and low molecular weight compounds, which favour colour stabilisation reactions and lead to an increase in colour intensity from red/violet to brick red (orange) tones which become more stable over time (Atanasova, Fulcrand, Cheynier, & Moutounet, 2002; Fernandes et al., 2017; Oliveira, Mateus, & de Freitas, 2017). These reactions are favoured by the presence of oxygen, thus improving the quality of the final wine (Atanasova, Fulcrand, Cheynier, & Moutounet, 2002; Pérez-Prieto, López-Roca, Martínez-Cutillas, Pardo Mínguez, & Gómez-Plaza, 2002; Sánchez-Gómez, del Alamo-Sanza, Martínez-Martínez et al., 2020).

The barrel plays a fundamental role in the properties acquired by the wine. In addition to the type and toasting of the wood from which it is made, the rate of oxygen transmission is a very important property as it affects the evolution of the wine (Prat-García et al., 2021; Sánchez-Gómez, del Alamo-Sanza, Martínez-Martínez et al., 2020). In general, the barrel is chosen according to the origin, grain and toasting degree of the wood. The type of grain has always been related to the porosity of the wood. However, Nevares et al. (2019) demonstrated that there is no direct relationship between the grain of the wood and its capacity to transfer oxygen, since planks with different oxygen transmission rates were found within the group of fresh staves classified in different grain categories after measuring 267 pieces. Some cooperages

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offer the possibility to choose barrels built with graded wood (Oakscan, Radoux, France) to provide more or less phenolic content to their wines (Michel, 2012; Michel et al., 2011). The UVaMOX group has developed a tool that allows the construction of barrels with different oxygen transmission rates (OTR) using staves classified according to their OTR based on their anatomical characteristics (Nevares & del Alamo-Sanza, 2015; Nevares et al., 2019). It has been shown that barrels that oxygenate twice as much as others can be built from the same batch of French oak (Prat-García, Nevares, Martínez-Martínez, & delAlamo-Sanza, 2020), and the resulting wines differ (Prat-García et al., 2021; Sánchez-Gómez, del Alamo-Sanza, & Nevares, 2020; Sánchez-Gómez, del Alamo-Sanza, Martínez-Martínez et al., 2020). The study of the same wine aged in these barrels indicated that the wine from the highly oxygenated barrels acquired a greater capacity to consume oxygen (Sánchez-Gómez, del Alamo-Sanza, Martínez-Martínez et al., 2020). In addition, the wood from French oak barrels classified as HOTR was observed to have a different volatile composition to that from LOTR barrels, a result that was evident in the wine aged in these (Sánchez-Gómez, del Alamo-Sanza, & Nevares, 2020). Specifically, the same red wine after 3 months of ageing in LOTR barrels had a higher content of furan compounds, acetovanillone and volatile phenolic aldehydes, than when aged in HOTR barrels. In relation to the evolution of anthocyanins, it was found that after 6 months of ageing the wine from high oxygenation barrels maintained a higher level of monomeric anthocyanins, showing statistically significant differences at 12 months (Prat-García et al., 2021).

Several studies have focused on differentiating the type of barrel used for ageing wines in barrels according to their chemical parameters (Basalekou, Pappas, Tarantilis, Kotseridis, & Kallithraka, 2017; del Alamo, Bernal, & Gomez-Cordoves, 2000; delÁlamo Sanza, Nevares Domínguez, & Merino García, 2004; Rosso, Panighel, Vedova, Stella, & Flamini, 2009; Watrelot, Badet-Murat, & Waterhouse, 2018). Other studies have focused on monitoring the ageing time of red wines using different physico-chemical parameters (Astray et al., 2019). This work is part of a project focused on studying the effect of ageing the same red wine when aged in barrels made of French oak but with different oxygenation rates. The formation of new pigments has been studied by Prat-García et al. (2021) and this study presents the low molecular weight phenol, los global phenolic parameters, phenolic indexes, colour characteristics and chemical age evolution of the same red wine aged in *Quercus petraea* Liebl. wooden barrels with different OTRs throughout an ageing period of 12 months (with sampling at 3, 6, 9 and 12 months). The use of barrels with different oxygen transmission rates allowed us to obtain wines with different chromatic characteristics and different phenolic compositions.

## 2. Materials and methods

### 2.1. Barrels and wine ageing

Eight 225-litre French oak (*Quercus petraea* Liebl.) barrels were used, made from staves from the same 3-year-old naturally dried batch, which were previously classified according to their OTR (Martínez-Martínez, delAlamo-Sanza, & Nevares, 2019). The barrels were built by the INTONA S.L. cooperage (Navarra, Spain) using medium toasting from room temperature up to 180 °C and maintained at this temperature for 40 min, 20 min on each side. Prior to that, the barrels were submitted to a 30 min preheating fire process for bending, while the exterior surface of the barrel was periodically sprayed with water to relax the wood fibres. The temperature outside the barrel was measured and values were no higher than 60 °C. The OTR characterization of the barrels that had been constructed was performed by evaluating the amount of atmospheric oxygen entering the barrel. For this purpose, another 8 barrels constructed in the same way as those used to age the wine and following the same methodology (del Alamo-Sanza & Nevares, 2014), were filled with deoxygenated model wine, which does not consume oxygen, and by means of submersible probes incorporated by the barrel bung, the accumulation of oxygen in the model wine was measured allowing to quantify the rate of oxygen ingress. This characterization of barrels indicated that HOTR barrels (OTR $= 1.29 \pm 0.06$ hPa/barrel.day), together with the initial degassing of the wood, would provide the wine with more than twice as much oxygen as LOTR barrels (OTR $= 0.68 \pm 0.13$ hPa/barrel.day) (Prat-García et al., 2020).

A young red wine produced on an industrial scale in 2017 in a winery of a Spanish denomination of origin (DO), Ribera del Duero, was used and named initial wine. The chemical parameters of the initial wine were total acidity 4.56 g/L (expressed as tartaric acid), pH 3.91, volatile acidity 0.48 g/L (expressed as acetic acid), reducing sugar 1.40 g/L and other sugars 1.21 g/L, alcohol concentration 15.16%, colour intensity 15, and total polyphenol index 61, free $SO_2$ 18 mg/L and total $SO_2$ 42 mg/L. The barrels were filled with wine and stored in a barrel room at the University of Valladolid under controlled conditions of temperature and humidity (15-16 °C and 70–75%, respectively). The wine was aged in the barrels for 12 months. The wine was sulphited after 6 months at 30 mg/L free $SO_2$ level. Sampling was carried out under nitrogen atmosphere at 3, 6, 9 and 12 months. All analyses were carried out in duplicate.

### 2.2. Wine analyses

#### 2.2.1. Oenological parameters

Alcohol concentration (AC %, L ethanol/100L wine), titratable acidity (TA, g/L of tartaric acid), pH, volatile acidity (VA, expressed as g/L of acetic acid), reducing sugar (RS, expressed as g/L), glucose and fructose (G+F, expressed as g/L), with the difference between RS and G+F reflecting the content in other reducing sugars such as hexoses and pentoses (OS, expressed as g/L), free and combined $SO_2$ (F–$SO_2$ and C–$SO_2$, expressed as mg/L according to OIV-MA-AS323-04B, iron (Fe, expressed as mg/L) and copper (Cu, expressed as mg/L) were measured in the initial wine and after 3, 6, 9 and 12 months in barrels following the methods established by International Organization of Vine and Wine (OIV, 2019). Major volatile compounds (acetaldehyde, ethyl acetate, methanol, 1-propanol, isobutanol, 2-methyl-1-butanol, 3-methyl-1-butanol), expressed in mg/L of the corresponding standard, were also analysed following the method described in Pérez-Magariño et al. (2019) using a gas chromatograph with a flame ionization detector (GC-FID).

#### 2.2.2. Phenolic parameters and colour

The total phenols (TP), low polymerized phenols (LPP) and high polymerized phenols (HPP) as mg/L of gallic acid, total anthocyanins (ACY, as mg/L of malvidin-3-O-glucoside) and tannins (TAN, as g/L of cyanidin chloride) were analysed according to the methods described elsewhere (del Alamo Sanza, Fernández Escudero, & De Castro Torío, 2004). The Ionization index (IoIn) measures the proportion of coloured and uncoloured anthocyanins. It is based on the difference in the effect of $SO_2$ bleaching on wine colour at normal wine pH and at a pH of 1 and indicates the anthocyanin percentage associated with wine colour. The Gelatin index (GIn) measures the reactivity of wine tannins with gelatin and it is used as an indication of astringency or dry tannin and the Ethanol index (EtIn) is the tannin percentage associated with wine polysaccharides. Both were determined according to Ribéreau-Gayon, Glories, Maujean, and Dubourdieu (2006) method. Chemical age was defined according to the parameters (i), (ii), (i/ii), (α) and (αα) using Somers & Evans, 1977 method: (i) indicates the relation between polymeric pigments and wine ACYs content; (ii) reflects the relation between polymeric pigments and wine anthocyanin measured in their ion flavilium forms; (i/ii) is the relation of wine anthocyanins in the form of the flavilium ion with respect to wine anthocyanins; (α) represents the percentage of anthocyanins that are found as flavilium ion; and (αα) is the percentage of anthocyanins as ion flavilium after removal of the decolourising effect of $SO_2$.

Colour parameters were determined by a direct measurement of wine absorbance at 420, 520 and 620 nm in a 1 mm optical path length Quartz cuvette with PerkinElmer's LAMBDA 25 UV/vis Spectrophotometer (Waltham, MA, USA). CI (colour intensity), hue (T) and %dA (percentage of free or combined ion flavilium anthocyanin), red, yellow, and blue percentages were also calculated following the method described elsewhere (del Alamo Sanza, Fernández Escudero, & De Castro Torío, 2004).

#### 2.2.3. *Individual low molecular weight phenols (LMWP), catechin and epicatechin*

The analysis of low molecular weight compounds (hydroxybenzoic acids, including gallic acid, protocatechuic acid, vanillic acid, syringic acid and gentisic acid; hydroxycinnamic acids including caffeic acid and *p*-coumaric acid; aldehydes, including *p*-hydroxybenzoic aldehyde and vanillin), catechin and epicatechin were analysed following the method described elsewhere del Alamo, Casado, Hernández, and Jiménez (2004). Samples and standards were extracted according to the mentioned method and each compound was identified by comparing their peak retention times to those previously obtained by the standards and they were quantified by means of specific calibration curves.

### 2.3. *Statistical analyses*

The software package Statistica 64 (StatSoft, Inc. USA) was used for the ANOVA, regressions and principal component analysis. The ANOVA was performed according to Fisher test ($p < 0.05$) to discover any statistically significant differences between the wine aged in the 4 LOTR and in the 4 HOTR barrels. The regression equations between the oxygen doses and parameter variance were carried out to observe the significant differences that can be attributed to the different oxygen doses received and consumed by the wines in both types of barrels. The principal component analysis was carried out to obtain a global view of the influence that the OTR of the barrels had on the characteristics of the wines during ageing.

## 3. Results and discussion

It is important to bear in mind that the wine aged in the 8 barrels was the same and that the barrels were made of French oak from the same batch and origin and following the same cooperage protocol. Therefore, the existing differences can be attributed to half of the barrels dosing more than twice as much oxygen to the wine as the other half, as a consequence of the wood's different anatomical characteristics.

**Table 1**
Oenological parameters of the wines aged during 12 months in barrels with low oxygen transmission rate (LOTR) and high oxygen transmission rate (HOTR).

| Time | 0 months | 3 months | | 6 months | | 9 months | | 12 months | |
|---|---|---|---|---|---|---|---|---|---|
| Barrels | Initial wine | 4 LOTR barrels | 4 HOTR barrels | 4 LOTR barrels | 4 HOTR barrels | 4 LOTR barrels | 4 HOTR barrels | 4 LOTR barrels | 4 HOTR barrels |
| AC | 15.16 ± 0.12 | 15.17 ± 0.03 a | 15 .18 ± 0.01 a | 15 .08 ± 0.02 a | 15 .08 ± 0.02 a | 15 .17 ± 0.02 a | 15 .19 ± 0.01 a | 15 .25 ± 0.01 a | 15 .26 ± 0.01 a |
| TA | 4.56 ± 0.01 | 4.67 ± 0.01 a | 4 .69 ± 0.01 b | 4 .95 ± 0.02 a | 4 .95 ± 0.04 a | 4 .91 ± 0.02 a | 4 .93 ± 0.04 a | 4 .66 ± 0.03 a | 4 .72 ± 0.02 b |
| pH | 3.91 ± 0.01 | 3.98 ± 0.02 a | 4 .00 ± 0.01 a | 3 .85 ± 0.01 a | 3 .85 ± 0.01 a | 3 .83 ± 0.00 a | 3 .83 ± 0.01 a | 3 .84 ± 0.01 b | 3 .83 ± 0.00 a |
| VA | 0.48 ± 0.01 | 0 .61 ± 0.01 a | 0 .63 ± 0.04 a | 0 .70 ± 0.03 a | 0 .72 ± 0.01 a | 0 .73 ± 0.04 a | 0 .72 ± 0.01 a | 0 .76 ± 0.02 a | 0 .76 ± 0.02 a |
| RS | 1.4 ± 0.05 | 1.43 ± 0.05 a | 1 .40 ± 0.00 a | 1 .58 ± 0.05 a | 1 .58 ± 0.05 a | 1 .60 ± 0.00 a | 1 .65 ± 0.06 a | 1 .55 ± 0.06 a | 1 .58 ± 0.05 a |
| G+F | 0.19 ± 0.05 | 0.24 ± 0.01 a | 0 .24 ± 0.01 a | 0 .38 ± 0.05 a | 0 .38 ± 0.03 a | 0 .43 ± 0.01 a | 0 .45 ± 0.02 b | 0 .35 ± 0.01 a | 0 .35 ± 0.01 a |
| OS | 1.21 ± 0.05 | 1.18 ± 0.05 a | 1 .17 ± 0.01 a | 1 .20 ± 0.05 a | 1 .20 ± 0.03 a | 1 .18 ± 0.01 a | 1 .20 ± 0.04 a | 1 .20 ± 0.05 a | 1 .23 ± 0.04 a |
| F–$SO_2$ | 18 ± 0.5 | 12.50 ± 0.58 a | 13.25 ± 0.50 b | 2.00 ± 0.00 a | 2.00 ± 0.00 a | 25 .50 ± 3.00 a | 27 .50 ± 0.58 b | 18.00 ± 4.24 a | 20 .75 ± 0.96 a |
| C–$SO_2$ | 24 ± 0.5 | 10.00 ± 1.15 b | 8 50 ± 1.00 a | 11 .50 ± 0.58 a | 12 .25 ± 0.50 b | 38 .75 ± 2.50 a | 42 .25 ± 1.26 b | 34 .75 ± 4.72 a | 39 .75 ± 0.96 b |
| Fe | 1.20 ± 0.01 | 1.03 ± 0.05 a | 1 .00 ± 0.00 a | 0 .88 ± 0.05 a | 0 .85 ± 0.06 a | 0 .99 ± 0.03 a | 0 .98 ± 0.04 a | 1.00 ± 0.00 a | 0 .95 ± 0.10 a |
| Cu | 0.12 ± 0.01 | 0.15 ± 0.01 b | 0 .13 ± 0.01 a | 0 .13 ± 0.01 a | 0 .14 ± 0.01 a | 0 .22 ± 0.01 a | 0 .20 ± 0.01 a | 0 .14 ± 0.01 b | 0 .11 ± 0.01 a |
| Acetaldehyde | 11.98 ± 0.10 | 12.00 ± 0.82 a | 11 .50 ± 1.00 a | 10.00 ± 0.82 a | 10.00 ± 0.82 a | 31 .50 ± 3.00 a | 34 .50 ± 2.08 a | 34 .50 ± 7.37 a | 36 .25 ± 9.74 a |
| Ethyl acetate | 47.12 ± 0.11 | 48.75 ± 6.50 a | 52 .25 ± 9.64 a | 70.00 ± 1.83 b | 64 .50 ± 0.58 a | 84 .25 ± 2.63 a | 85 .75 ± 1.50 a | 60.00 ± 2.58 a | 62.00 ± 1.41 a |
| Methanol | 264.08 ± 1.22 | 271.50 ± 5.00 a | 268 .25 ± 1.26 a | 265 .75 ± 11.79 a | 273.00 ± 10.13 a | 251 .50 ± 3.32 a | 249 .50 ± 3.70 a | 226 .25 ± 2.06 b | 217 .50 ± 2.38 a |
| 1-Propanol | 27.51 ± 0.74 | 27.00 ± 0.18 a | 26 .98 ± 0.13 a | 26 .85 ± 2.20 a | 27 .075 ± 1.02 a | 32 .95 ± 0.85 a | 34 .58 ± 0.52 b | 28 .88 ± 0.46 a | 28 .88 ± 0.15 a |
| Isobutanol | 60.26 ± 0.25 | 58.58 ± 0.39 a | 59 .40 ± 0.96 a | 58 .86 ± 1.83 a | 57 .13 ± 2.10 a | 67 .85 ± 1.73 a | 71 .48 ± 1.17 b | 57 .33 ± 0.57 a | 43 .95 ± 26.64 a |
| 2-Methyl-1-butanol | 69.93 ± 1.12 | 62.30 ± 1.40 a | 63 .88 ± 1.47 a | 60 .85 ± 1.76 a | 59 .43 ± 2.71 a | 70 .15 ± 1.94 a | 74 .05 ± 1.27 b | 58 .55 ± 1.20 a | 58 .65 ± 0.68 a |
| 3-Methyl-1-butanol | 244.35 ± 1.52 | 234.50 ± 2.61 a | 236 .60 ± 2.35 a | 247 .45 ± 9.25 a | 239 .38 ± 7.56 a | 277 .88 ± 6.45 a | 292 .10 ± 4.07 b | 239 .65 ± 3.43 a | 240 .80 ± 2.27 a |
| Total higher alcohols | 392.97 ± 5.03 | 382.38 ± 4.11 a | 386 .85 ± 4.17 a | 394 .01 ± 9.23 a | 321 .45 ± 114.46 a | 448 .83 ± 10.95 a | 472 .20 ± 6.94 b | 384 .40 ± 4.53 a | 372 .28 ± 27.63 a |

AC: alcohol concentration (%v/v); TA: titratable acidity (g/L of tartaric acid); VA: volatile acidity (g/L of acetic acid); RS: reducing sugar (g/L); G+F: glucose and fructose (g/L); OS: other sugars (g/L); F–$SO_2$: free $SO_2$ (mg/L); C–$SO_2$ combined $SO_2$ (mg/L), Fe: iron (mg/L): Cu: copper (mg/L). Major volatile compounds (acetaldehyde, ethyl acetate, methanol, 1-propanol, isobutanol, 2-methyl-1-butanol, 3-methyl-1- butanol), expressed in mg/L of the corresponding standard. Data are mean values ± standard deviation (n = 8, consisting in four barrel replicates for each treatment and two technical replicates for each barrel replicate); For the same row, different letters in different columns indicate significant differences at that time between the wine of the LOTR and HOTR barrels, according to Fisher's LSD test ($p < 0.05$).

### 3.1. Wine oenological parameter evolution

Table 1 shows the basic parameters of the initial wine and of the wines aged in barrels with different oxygenation rates (4 LOTR and 4 HOTR) throughout the 12 months of ageing, including comparative ANOVA after 3, 6, 9 and 12 months. These parameters reflect the quality of the wine (volatile acidity or alcohol concentration) and its stability (total acidity and pH), these being related to the extractive capacity of the wood components (Ortega-Heras, González-Sanjosé, & González-Huerta, 2007). There were no statistically significant differences in the basic parameters of wines aged in high or low oxygenation barrels in any of the samples. The evolution of these parameters in the wines, due to the ageing time, was as expected. The alcoholic concentration of the wines aged in the 8 barrels increased after 12 months of ageing by 0.6 and 0.7% in the wines from the LOTR and HOTR barrels, respectively, a logical result as water evaporates faster than ethanol. The total acidity of the wines increased at the beginning of ageing up to 6 months, then remained constant up to 9 months before decreasing at 12 months. Volatile acidity increased during the 12 months of ageing by 48% with respect to the initial content. This result is usual in barrel ageing processes and is caused by the transfer of compounds from the wood acetyl groups (García-Moreno et al., 2021; Vivas, Lonvaud-Funel, & Glories, 1995). During ageing an increase in residual sugars was observed, which is partly due to hydrolysis of anthocyanins containing glucose in their molecule, as well as to the degradation of certain polysaccharides and even to the degradation of wood compounds (del Alamo et al., 2000). The results of free sulphur in the wines after 6 months of ageing in both types of barrels were below 5 mg/L (2 mg/L, see Table 1), a very low level taking into account that the method has an error of 7 mg/L (Zoecklein, Fugelsang, Gump, & Nury, 1995), so $SO_2$ addition was necessary. The addition was carried out, hence the increase observed in the values of the wines after 9 months. The decrease in total $SO_2$ was 50% in the first 3 months and 65% after 6 months. This loss especially reflects the drop in combined $SO_2$ in the first 3 months and in free $SO_2$ after 6 months. The loss of total $SO_2$ is due to interaction with wine compounds and is common in wine maturation processes, and also causes changes in the free and combined sulphur dioxide balance. Acetaldehyde is the main product of ethanol oxidation and plays an important role in the formation of adducts linked by an ethyl bridge, important in the formation of tannin-tannin and tannin-anthocyanin complexes, because they are more stable pigments over time (Es-Safi, Cheynier, & Moutounet, 2002; Liu, Zhang, Shi, Duan, & He, 2019; Sheridan & Elias, 2016). No significant differences were found between wines aged in high and low oxygenation barrels, a higher acetaldehyde content was found in wines aged in HOTR barrels at 9 and 12 months has been observed. This higher concentration is probably linked to the fact that these wines received twice as much oxygen.

### 3.2. Relationship between oxygen uptake and wine analysis

The level of oxygen reaching the wines aged in HOTR barrels was higher than that received by the wines in LOTR barrels. Therefore, the relationship between the evolution of the compounds studied and the oxygen received by the wines during the year of barrel ageing was studied. The oxygen dosed by the barrels was studied by Prat-García et al. (2020) in 4 HOTR barrels and 4 LOTR barrels made from the same wood and in the same batch as the barrels used for the ageing of the wine described, since 8 HOTR barrels and 8 LOTR barrels were built. The study of the relationship between oxygen and the evolution of the compounds in the wine was carried out by averaging the oxygen dosed by the 4 HOTR barrels and the average of each compound in the wine from the 4 HOTR barrels; the same was done for the 4 LOTR barrels. Table 2 presents only the regressions showing statistically significant differences ($p < 0.1$) between the LOTR and HOTR barrels, i.e., there was a statistically significant difference in the slope of the regression of the variation of the parameters ACY, HPP, i, A420, A620, gallic acid, protocatechuic acid, *p*-coumaric acid, caffeic acid, *p*-hydroxybenzoic aldehyde and epicatechin in the wine and oxygen received in the LOTR and HOTR barrels. As mentioned above, a significance of 90% was considered since the wine aged in the 8 barrels was the same and the barrels were made of French oak wood, of the same origin, and following the same cooperage procedure. Therefore, significant differences were analysed, which can basically be attributed to the different amounts of oxygen received and consumed by the wines in both types of barrels, and also to the anatomy of the wood itself and its variability as a natural material, which affects all barrels.

**Table 2**
Regression equations (y = a + b x) obtained between the accumulative oxygen doses (x) and parameter variation (y) in wines aged during one year in LOTR and HOTR barrels. The average of the oxygen dosed by the 4 HOTR and 4 LOTR barrels at 3, 6, 9 and 12 months and the average of each compound in the wine from the 4 HOTR and 4 LOTR barrels were used.

| | H-OTR barrels | | L-OTR barrels | | |
|---|---|---|---|---|---|
| | a | b | a | b | p-level* |
| ACY | 0.2273 | −0.0506 | 0.5270 | −0.2151 | 0.0009 |
| HPP | 0.1441 | −0.0768 | 0.2536 | −0.1812 | 0.0913 |
| i | −0.2017 | 0.0535 | −0.5143 | 0.2203 | 0.0159 |
| A 420 nm | 0.0800 | −0.0152 | 0.1235 | −0.0455 | 0.0899 |
| A 620 nm | 0.1569 | −0.0287 | 0.2633 | −0.0933 | 0.0210 |
| Gallic acid | −0.6297 | 0.1538 | −1.2798 | 0.6455 | 0.0149 |
| Protocatechuic acid | −0.0872 | 0.2611 | −0.6443 | 0.8404 | 0.0837 |
| Coumaric acid | −0.3624 | 1.2946 | −0.1491 | 3.2535 | 0.0474 |
| Caffeic acid | 3.3243 | 1.6909 | 1.0159 | 5.1582 | 0.0085 |
| *p*-hydroxybenzoic aldehyde | 0.7320 | 0.2464 | −0.0937 | 0.9406 | 0.0075 |
| Epicatechin | −0.8425 | 0.0622 | −0.8719 | 0.1741 | 0.0823 |

ACY: total anthocyanins; HPP: high polymerized phenols; i: relation between polymeric pigments and wine anthocyanin content. *p-level according to differences in the slope between LOTR and HOTR regression.

Fig. 1 presents the regressions obtained between the average oxygen received by the wines from the 4 LOTR and 4 HOTR barrels at each ageing time (3, 6, 9 and 12 months) and the variation of each parameter at each time with respect to the initial value in the wine (Δ). The decrease in total anthocyanins (ACY) and high polymerized phenols (HPP) is closely related to the oxygen received by the wines, which was practically the oxygen consumed since the dissolved oxygen levels in the wines from the barrels were below 50 ppb (Nevares & delÁlamo, 2008).

Wines from LOTR barrels receiving less oxygen were found to suffer greater ACY loss throughout the ageing process with a final result of 30% less than in the initial wine (Table 3). Thus, Fig. 1a shows that anthocyanin loss according to the dosed oxygen in wines from HOTR barrels was four times lower, slope −0.0506, compared to wines aged in LOTR barrels with a slope of −0.2151 (Table 2) and with a higher variation in the first 6 months. Oxygen plays a fundamental role in the reactions where anthocyanins are involved, with many studies showing that adequate amounts of oxygen lead to an evident modification and stabilisation of colour. The decrease of these anthocyanins is one of the most important processes occurring during ageing and is caused by the transformation of these compounds due to oxidation, condensation, and polymerisation reactions. Previous studies have been carried out on how the use of different wood species, origins, number of uses of barrels, as well as different doses of oxygen provided by micro-oxygenation or different micro-oxygenation systems, or the different characteristics of the initial wine can affect anthocyanins. Thus, del Alamo-Sanza, Nevares, Gallego, Fernández de Simón, and Cadahía (2010) in a work carried out with oak products and on-demand micro-oxygenation, reported that wines requiring the least amount of oxygen are those that suffer the greatest loss of anthocyanins.

As mentioned above, there are no studies that evaluate the effect of oxygen on wine during ageing in barrels, as this is unknown and only the quantity of dissolved oxygen remaining in the wine is known, i.e., the

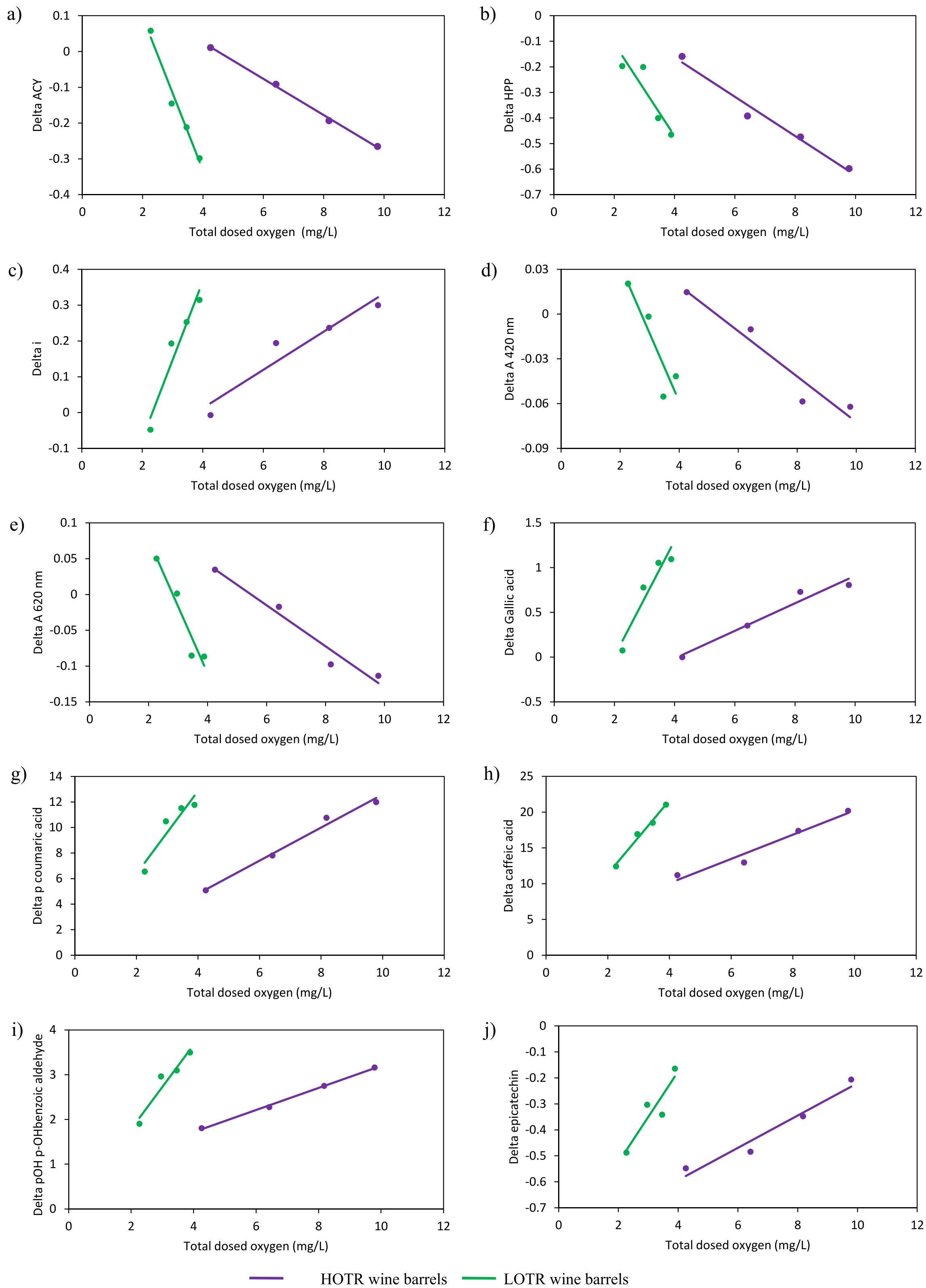


**Fig. 1.** Graphic representation of the regression equations obtained between the accumulative oxygen doses and parameter variation in wines aged in LOTR and HOTR barrels. Each point represents the average of the wine samples collected from the 4 LOTR and 4 HOTR oak barrels and the oxygen dosage of the 4 LOTR and 4 HOTR oak barrels.

oxygen that the wine has not consumed and which is usually between 20 and 50 μg/L (Nevares & delÁlamo, 2008). The available studies on the effect of oxygen on the properties of wines describe results from experiments in which oxygen was added to wines with a micro-oxygenator and wood in the form of alternatives, a process significantly different from barrel ageing. Thus, the work of Cano-López, López-Roca, Pardo-Minguez, and Gómez Plaza (2010) showed greater losses of monomeric anthocyanins in wines with MOX than in wines aged in

**Table 3**
Analysis results of global phenolic parameters. index. Colour characteristics and chemical age in the wines aged during 12 months in barrels with low oxygen transmission rate (LOTR) or high oxygen transmission rate (HOTR).

| Time | 0 months | 3 months | | 6 months | | 9 months | | 12 months | |
|---|---|---|---|---|---|---|---|---|---|
| Barrels | Initial wine | 4 LOTR barrels | 4 HOTR barrels | 4 LOTR barrels | 4 HOTR barrels | 4 LOTR barrels | 4 HOTR barrels | 4 LOTR barrels | 4 HOTR barrels |
| ***Global phenolic parameters*** | | | | | | | | | |
| TP | 2046.60 ± 46.32 | 1941.60 ± 108.00 a | 1955.35 ± 82.18 a | 2295.10 ± 46.16 b | 2172.85 ± 78.49 a | 1511.35 ± 43.26 a | 1500.60 ± 56.54 a | 1655.35 ± 313.12 a | 1438.10 ± 326.34 a |
| LPP | 742.61 ± 12.35 | 894.55 ± 53.99 a | 859.10 ± 68.32 a | 1252.85 ± 92.74 a | 1380.73 ± 68.77 b | 730.10 ± 39.72 a | 815.67 ± 42.47 b | 958.56 ± 120.98 a | 914.10 ± 192.14 a |
| HPP | 1340.02 ± 24.86 | 1047.05 ± 156.78 a | 1096.25 ± 83.28 a | 1042.25 ± 115.16 b | 792.13 ± 65.97 a | 781.25 ± 79.21 b | 684.93 ± 63.84 a | 696.79 ± 309.22 a | 524.00 ± 398.32 a |
| ACY | 570.06 ± 1.85 | 603.11 ± 7.23 a | 576.08 ± 40.43 a | 487.23 ± 10.51 a | 517.68 ± 11.51 b | 449.20 ± 17.84 a | 459.71 ± 6.57 a | 400.09 ± 20.62 a | 418.69 ± 4.27 b |
| TAN | 2.76 ± 0.04 | 2.26 ± 0.12 a | 2.48 ± 0.22 b | 2.51 ± 0.13 a | 2.92 ± 0.22 b | 2.63 ± 0.17 b | 2.40 ± 0.24 a | 2.75 ± 0.09 a | 2.76 ± 0.11 a |
| ***Index*** | | | | | | | | | |
| IoIn | 25.84 ± 0.26 | 18.86 ± 6.40 a | 17.48 ± 3.78 a | 33.58 ± 1.07 a | 32.91 ± 0.78 a | 28.62 ± 2.89 b | 25.32 ± 1.50 a | 11.83 ± 0.75 a | 11.49 ± 0.63 a |
| EtIn | 17.32 ± 0.58 | 16.63 ± 7.20 a | 17.30 ± 3.47 a | 41.51 ± 1.94 a | 45.63 ± 1.94 b | 26.10 ± 2.30 a | 27.14 ± 2.50 a | 35.03 ± 8.82 a | 29.46 ± 1.89 a |
| Gln | 55.94 ± 1.69 | 39.70 ± 11.41 a | 35.83 ± 9.42 a | 47.46 ± 6.85 a | 55.89 ± 6.79 b | 52.49 ± 4.05 a | 55.03 ± 2.24 a | 50.16 ± 3.67 a | 55.61 ± 7.25 a |
| ***Chemical age*** | | | | | | | | | |
| α | 16.49 ± 0.10 | 17.21 ± 0.31 a | 18.90 ± 1.25 b | 21.01 ± 0.64 a | 20.32 ± 0.80 a | 17.22 ± 1.01 a | 17.60 ± 0.98 a | 13.89 ± 1.42 a | 14.43 ± 0.60 a |
| αα | 20.22 ± 0.14 | 20.65 ± 0.66 a | 19.97 ± 1.62 a | 21.93 ± 0.55 b | 20.64 ± 0.50 a | 25.74 ± 0.44 a | 27.51 ± 2.19 b | 16.94 ± 2.38 a | 18.27 ± 0.66 a |
| i | 0.443 ± 0.00 | 0.422 ± 0.00 a | 0.440 ± 0.02 a | 0.528 ± 0.01 a | 0.529 ± 0.01 a | 0.555 ± 0.01 a | 0.548 ± 0.01 a | 0.582 ± 0.02 a | 0.576 ± 0.01 a |
| ii | 0.127 ± 0.00 | 0.122 ± 0.00 a | 0.124 ± 0.00 a | 0.173 ± 0.01 a | 0.169 ± 0.00 a | 0.209 ± 0.00 a | 0.212 ± 0.01 a | 0.169 ± 0.01 a | 0.175 ± 0.01 a |
| i/ii | 3.49 ± 0.01 | 3.46 ± 0.13 a | 3.55 ± 0.16 a | 3.06 ± 0.06 a | 3.14 ± 0.05 a | 2.66 ± 0.03 a | 2.59 ± 0.13 a | 3.47 ± 0.32 a | 3.28 ± 0.07 a |
| ***Colour characteristics*** | | | | | | | | | |
| CI | 18.18 ± 0.09 | 18.34 ± 0.83 a | 18.13 ± 0.44 a | 18.20 ± 0.43 a | 17.94 ± 0.11 a | 16.51 ± 0.39 a | 16.36 ± 0.27 a | 16.84 ± 0.57 a | 16.40 ± 0.27 a |
| T | 0.76 ± 0.00 | 0.79 ± 0.03 a | 0.77 ± 0.08 a | 0.74 ± 0.00 a | 0.74 ± 0.00 a | 0.85 ± 0.01 a | 0.86 ± 0.02 a | 0.68 ± 0.06 a | 0.69 ± 0.01 a |
| %420 | 37.31 ± 0.02 | 37.75 ± 0.13 a | 37.96 ± 0.15 b | 37.21 ± 0.05 a | 37.42 ± 0.01 b | 38.82 ± 0.31 a | 39.03 ± 0.09 a | 38.61 ± 0.34 a | 38.79 ± 0.08 a |
| %520 | 48.82 ± 0.01 | 47.83 ± 0.66 a | 47.65 ± 0.33 a | 48.91 ± 0.09 b | 48.76 ± 0.02 a | 47.21 ± 0.18 a | 47.06 ± 0.09 a | 47.71 ± 0.22 a | 47.59 ± 0.03 a |
| %620 | 13.87 ± 0.01 | 14.42 ± 0.68 a | 14.39 ± 0.19 a | 13.88 ± 0.12 a | 13.82 ± 0.03 a | 13.97 ± 0.15 a | 13.91 ± 0.10 a | 13.68 ± 0.13 a | 13.63 ± 0.07 a |
| %dA | 47.58 ± 0.02 | 45.48 ± 1.34 a | 45.06 ± 0.68 a | 47.48 ± 0.21 b | 47.46 ± 0.12 a | 44.09 ± 0.37 b | 43.76 ± 0.18 a | 45.20 ± 0.45 a | 44.93 ± 0.09 a |

TP: total phenols (mg/L); LPP: low polymerized phenols (mg/L); HPP: high polymerized phenols (mg/L); ACY: total anthocyanins (mg/L); TAN: tannins (g/L); IoIn: ionization index; EtIn: ethanol index; Gln: gelatin index; α: percentage of anthocyanins that are found as flavilium ion; αα percentage of anthocyanins as ion flavilium after removal of the decolourising effect of SO2; i: relation between polymeric pigments and wine anthocyanin's content; ii: relation between polymeric pigments and wine anthocyanin measured in their ion flavilium forms; i/ii: relation of wine anthocyanins in the form of flavilium ion with respect to wine anthocyanins; CI: colour intensity; T: hue; %dA: percentage of free or combined ion flavilium anthocyanin. Data are mean values ± standard deviation (n = 8, consisting in four barrel replicates for each treatment and two technical replicates for each barrel replicate)); For the same row, different letters in different columns indicate significant differences at that time between the wine of the LOTR and HOTR barrels, according to Fisher's LSD test ($p < 0.05$)..

barrels, and within those aged in barrels, greater losses of these compounds when the barrels were new. Or the work of Sánchez-Gómez, Alamo-Sanza, Martínez-Gil, & Nevares (2020) studying the effect of ageing in different Micro-Oxygenation Systems with oak wood showed that passive (Nevares & delÁlamo, 2008) and dynamic MOX in barrels attenuated the loss of monoglucoside anthocyanins compared to the rest of the ageing systems, whereas the active MOX system causing the greatest loss. Previous work by the group studying wine aged in these barrels showed that wines aged in HOTR barrels had more monomeric anthocyanins than wines aged in LOTR barrels, and contained different derived compounds, thus demonstrating that the amount of oxygen the wine received defined both the loss of anthocyanins and the formation of new pigments (Prat-García et al., 2021).

The variation of highly polymerized compounds (Fig. 1b) also showed a relationship with the oxygen content received. Wines from barrels allowing higher oxygen permeability showed a greater decrease in HPP, with a gradual decrease in delta over months, while wines aged in LOTR barrels showed the most marked decrease from 6 to 9 months. Fig. 1c shows the good correlation between the increase in polymeric pigments formed from anthocyanins (i) and the amount of oxygen received. Wines from LOTR barrels had a slope of 0.2203 compared to wines aged in HOTR barrels with a slope of 0.0535 (Fig. 1c). The slight decrease in (i) reached in the first 3 months in the LOTR barrel wines was possibly caused by losses due to precipitation, while they remained at practically the initial levels in the case of the HOTR barrel wines. The evolution of ACY and i coincided with that observed in the work of del Alamo-Sanza et al. (2010), who found that wines that consume less oxygen suffer a greater loss of anthocyanins together with a greater increase in the i parameter. The formation of new compounds involving anthocyanins makes wine colour more stable with less loss over time. In Fig. 1 d and e, the second half of the ageing period saw a further decrease in the absorbances at 420 nm and 620 nm which was related to the oxygen received by the wines and was significantly higher in the wines from the HOTR barrels. This was related to the higher anthocyanin content (Fig. 1a), lower i (Fig. 1c) and lower HPP (Fig. 1c) of the wines aged in HOTR and indicated that the wines receiving lower amounts of oxygen underwent higher condensation and polymerisation processes where anthocyanins were involved. Therefore, wines that received less oxygen suffered less colour loss, possibly due to the formation of more stable-coloured compounds (Chassaing et al., 2010; Dangles & Fenger, 2018; Prat-García et al., 2021).

The content of low molecular weight compounds and their transfer to the wine is related to the oxygen received by the wines during ageing in wood. Thus, the analysis of the relationship between the increase in content of low molecular compounds and the oxygen received by the wines indicated a statistically significant relationship in the wines from HOTR and LOTR barrels for gallic acid, protocatechuic acid, *p*-coumaric acid, caffeic acid, *p*-hydroxybenzoic aldehyde, as well as for the evolution of epicatechin (Table 4). The delta increase in the first 3 months was similar for wines aged in LOTR and HOTR barrels for gallic acid (Fig. 1f) and for *p*-hydroxybenzoic aldehyde (Fig. 1i). However, in the first 3 months there were already differences in the increase of *p*-coumaric acid and caffeic acid, depending on the barrel used, as the deltas were higher in the wines that had received lower amounts of oxygen (Fig. 1g and h). The increase in the content of these compounds was especially significant from 3 to 6 months in the wines aged in LOTR barrels, noting the large gap between the 3-month and 6-month points compared to the other sampling times. There was a significant transfer of the compounds from the wood to the wine during these months. However, the increase of these compounds in the HOTR barrel wines was similar between all the samplings, so the transfer was more gradual when the oxygen content received in the wines was higher. This meant that the wines had a higher content of these compounds after 6 months when the oxygen dose received was lower (LOTR barrels), and that they had even higher contents at 6 months than those found in the HOTR barrel wines at 9 months (Table 4). This can be observed in Fig. 1g and h in the case of *p*-coumaric acid and caffeic acid or in the case of gallic acid and *p*-hydroxybenzoic aldehyde where the concentrations were very similar in the case of gallic acid and *p*-hydroxybenzoic aldehyde (Fig. 1f and i).

After 6 months, the wines that received lower amounts of oxygen showed less significant increases. Therefore, after 12 months of barrel ageing, wines from LOTR barrels showed higher contents of gallic acid, *p*-hydroxybenzoic aldehyde and caffeic acid (Fig. 1f, i, and h), but because the transfer of wood compounds to HOTR-aged wines showed a more constant increase, with a similar increase every 3 months, by 12 months of ageing there were no significant differences depending on the oxygen dose received (Table 4). Wines aged in high oxygen permeability barrels also showed higher *p*-coumaric acid concentrations. (Fig. 1f). The decrease in epicatechin concentration was smaller in the first 3 months in the case of wines receiving a lower oxygen content (Fig. 1j).

### 3.3. Wine global phenolic parameters, index, chemical age and colour characteristics evolution

Table 3 shows the contents of the different parameters analysed in the wines from the 4 LOTR and 4 HOTR barrels during 12 months of ageing, including comparative ANOVA after 3, 6, 9 and 12 months. Statistically significant differences were found in the content of global phenolic parameters in wines aged in barrels with different oxygen transmission rates (LOTR and HOTR), especially at 6 months and 9 months. Thus, up to month 6 of ageing, TP increased by 12% in wines aged in LOTR barrels compared to 6% in wines aged in HOTR barrels. In the second half of the process the TP content decreased similarly in wines from all barrels, 28% in wines from LOTR barrels and 34% in wines from HOTR barrels. In general, the wines aged in LOTR barrels showed higher total phenol (TP) content (Fig. 1a), and higher high polymerized phenols (HPP) (Fig. 1 b), both significant at 6 months (Table 3), also showing a lower content of low polymerized phenols

**Table 4**
Concentration of low molecular weight phenol (LMWP) in the wines aged during 12 months in barrels with low oxygen transmission rate (LOTR) or high oxygen transmission rate (HOTR).

| Time | 0 months | 3 months | | 6 months | | 9 months | | 12 months | |
|---|---|---|---|---|---|---|---|---|---|
| Barrels | Initial wine | 4 LOTR barrels | 4 HOTR barrels | 4 LOTR barrels | 4 HOTR barrels | 4 LOTR barrels | 4 HOTR barrels | 4 LOTR barrels | 4 HOTR barrels |
| ***Low molecular weight phenol (LMWP)(mg/L)*** | | | | | | | | | |
| Gallic acid | 19.32 ± 0.04 | 20.79 ± 1.49 b | 19.32 ± 0.83 a | 34.39 ± 6.14 b | 26.15 ± 7.02 a | 39.73 ± 9.20 a | 33.46 ± 7.42 a | 40.52 ± 6.05 a | 34.95 ± 8.38 a |
| Protocatechuic acid | 0.92 ± 0.02 | 2.19 ± 0.25 a | 2.02 ± 0.10 a | 2.57 ± 0.21 b | 2.18 ± 0.22 a | 2.68 ± 0.21 a | 2.67 ± 0.20 a | 3.59 ± 0.07 b | 3.36 ± 0.29 a |
| Vanillin acid | 1.00 ± 0.01 | 4.34 ± 0.46 a | 4.06 ± 0.25 a | 5.88 ± 0.26 b | 5.13 ± 0.39 a | 5.59 ± 0.22 a | 5.27 ± 0.54 a | 5.87 ± 0.05 a | 5.65 ± 0.59 a |
| Syringic acid | 1.02 ± 0.02 | 5.70 ± 0.65 a | 5.66 ± 0.28 a | 9.19 ± 0.81 b | 7.25 ± 0.36 a | 7.47 ± 0.22 a | 7.27 ± 0.62 a | 8.11 ± 0.16 a | 7.71 ± 0.77 a |
| Gentisic acid | 2.05 ± 0.03 | 1.35 ± 0.19 a | 1.32 ± 0.06 a | 1.85 ± 0.25 b | 1.54 ± 0.03 a | 1.32 ± 0.20 a | 1.38 ± 0.16 a | 1.53 ± 0.15 a | 1.63 ± 0.19 a |
| *p*-coumaric acid | 0.58 ± 0.01 | 4.38 ± 0.36 b | 3.52 ± 0.53 a | 6.66 ± 1.30 b | 5.11 ± 0.85 a | 7.26 ± 0.52 a | 6.82 ± 1.15 a | 7.41 ± 0.21 a | 7.53 ± 0.83 a |
| Caffeic acid | 1.45 ± 0.05 | 19.48 ± 1.98 b | 17.69 ± 1.24 a | 26.02 ± 1.99 b | 20.23 ± 5.27 a | 28.29 ± 1.76 a | 26.65 ± 3.45 a | 31.97 ± 0.64 a | 30.74 ± 2.89 a |
| Vanillin | 0.50 ± 0.01 | 4.11 ± 0.63 a | 3.67 ± 0.23 a | 6.61 ± 0.29 b | 5.16 ± 0.39 a | 5.15 ± 0.40 a | 4.79 ± 0.58 a | 4.69 ± 0.21 a | 4.53 ± 0.42 a |
| *p*-hydroxybenzoic aldehyde | 0.20 ± 0.01 | 0.58 ± 0.09 a | 0.56 ± 0.03 a | 0.79 ± 0.11 b | 0.66 ± 0.03 a | 0.82 ± 0.08 a | 0.75 ± 0.07 a | 0.90 ± 0.02 b | 0.83 ± 0.07 a |
| ***Catechin and epicatechin (mg/L)*** | | | | | | | | | |
| Catechin | 114.72 ± 1.12 | 84.19 ± 6.98 b | 74.98 ± 5.10 a | 85.68 ± 10.50 b | 69.32 ± 14.96 a | 98.56 ± 11.27 a | 100.08 ± 19.56a | 89.08 ± 9.52 a | 87.96 ± 15,21 a |
| Epicatechin | 58.86 ± 1.01 | 30.12 ± 1.36 b | 26.59 ± 1.87 a | 40.99 ± 3.52 b | 30.33 ± 5.10 a | 38.75 ± 3.18 a | 38.37 ± 6.71 a | 49.18 ± 2,86 a | 46.69 ± 6.72 a |

Data are mean values ± standard deviation (n = 8, consisting in four barrel replicates for each treatment and two technical replicates for each barrel replicate); For the same row, different letters in different columns indicate significant differences at that time between the wine of the LOTR and HOTR barrels, according to Fisher's LSD test ($p < 0.05$).

(LPP) with significant differences at 6 and 9 months (Table 3).

This result indicates that wines from LOTR barrels underwent more polymerisation processes possibly caused by the interaction with wood components that led to the formation of new pigments, reflected in a higher colour intensity. The analysis of the French oak woods from which the barrels were made (Sánchez-Gómez, del Alamo-Sanza, & Nevares, 2020) indicated that the LOTR woods had higher concentrations of furfural, 5-methylfurfural and 5-hydroxymethylfurfural, these compounds being involved in the condensation of phenolic compounds (Atanasova, Fulcrand, Le Guernevé, Cheynier, & Moutounet, 2002; Es-Safi et al., 2002; Fernandes et al., 2017), which would explain the higher condensation and polymerisation in the wines aged in the LOTR barrels than in the HOTR ones. Thus, the ACY content of the wines during barrel ageing showed a very similar evolution to that obtained in other studies, with an initial increase followed by a continuous decrease during the 12 months of the study (Del Álamo Sanza et al., 2004). The increase observed in the first 3 months with respect to the initial wine was 6% in the wines from LOTR barrels and 1% in the wines from HOTR barrels. Subsequently, a decrease from 6 months onwards was more pronounced in the wines aged in LOTR barrels (Fig. 2c) with a significantly lower content than in those from HOTR barrels at 6 months (Table 3). The decrease was more marked between months 3 and 6 of ageing, with losses of 19% and 10% in LOTR and HOTR barrel wines, respectively, due to their participation in oxidation, condensation and polymerisation reactions as indicated above.

Regarding the nature of the anthocyanins, the ionization index (IoIn), which indirectly indicates the percentage of anthocyanins contributing to the colour of the wines, increased up to 6 months of ageing by 30% and 27% in the LOTR and HOTR barrel wines, respectively, compared to the initial value. After 6 months, a decrease in IoIn was more marked in the wines aged in HOTR barrels, and especially between months 6 and 9 of ageing: 23% compared to 14% in the LOTR barrel wines. In general, IoIn was higher in wines aged in LOTR barrels and significantly higher at 9 months of ageing (Table 3). Therefore, the wines aged in LOTR barrels, although they had a lower free anthocyanin content, participated in the red colour which was of greater importance.

On the other hand, the wines aged in HOTR barrels were found to suffer less loss of initial ACY content, presenting a higher content of anthocyanins: these do not participate in the red colour of the wine, as they are probably involved in compounds that provide brownish tones. Tannin content initially showed a decrease followed by an increase (Table 3), the former possibly caused by their participation in multiple reactions of precipitation, oxidation, depolymerisation and condensation with anthocyanins (Watrelot et al., 2018)' although they may also increase due to the hydrolysis of higher oligomers (Rubio-Bretón, Garde-Cerdán, & Martínez, 2018). The lower ACY and TAN content in LOTR barrel wines could be closely related to the higher condensation and/or polymerisation, these tannins and anthocyanins being involved in reactions to form more stable pigments such as tannin-tannin complexes and other tannin-anthocyanin reactions (Gómez-Plaza & Bautista-Ortín, 2019; Oliveira et al., 2017; Sheridan & Elias, 2016). The relationship between a higher content of total phenols and a higher degree of polymerisation with a lower content of tannins and anthocyanins in wines has been observed in wines by other authors (Martínez-Gil, del Alamo-Sanza, Nevares, Sánchez-Gómez, & Gallego, 2020).

Regarding the nature of tannins, in general it was observed that in most of the samples, and especially in the second half of ageing, wines aged in HOTR barrels presented higher levels of astringent tannins with higher Gln (Fig. 2d) and polysaccharide-bound tannins (EtIn), this being significant at 6 months (Table 3). These results are possibly due to the HOTR barrel wines being richer in flavan-3-ols, although other compounds that could precipitate saliva proteins were present (Perez-Prieto, De La Hera-Orts, López-Roca, Fernández-Fernández, & Gómez-Plaza, 2003). At 3 month a decrease in the gelatin index of 29% and 36% with respect to the initial value was found in LOTR and HOTR barrel wines, respectively, (Fig. 1d), which coincided with the decrease in tannin content observed during this period (Table 2). Subsequently, in the same way as the tannin content, the gelatine index increased in all wines, most significantly between months 3 and 6 and in HOTR barrel wines with an increase of 56% compared to 20% in LOTR barrel wines. Moreover, HOTR barrel wines showed the highest EtIn, which reflected the tannins associated with the polysaccharides, suggesting that these were small tannins that, when bound to polysaccharides, prevented the formation of high molecular weight tannins. This result agreed with the lower involvement of tannins in condensation and polymerisation reactions in HOTR barrel wines. The highest ethanol index in all wines was observed at 6 months, being more marked in HOTR barrel wines, with a value 263% higher than the initial value, while in LOTR barrel wines this value was 239%.

Parameters α and αα give us an estimate of the percentage of anthocyanins found as flavilium ion (α) and the percentage after removing the decolourising effect of $SO_2$ (αα). It was found that the wines that received the highest amount of oxygen, in general, showed the highest percentage of anthocyanins as flavilium ion (Table 2) and these also showed the highest total anthocyanin content (Fig. 2c) probably due to less interaction with the compounds released by the wood. The wines showed the lowest α and αα values at 12 months as expected since the flavylium form is the most unstable and therefore participates in oxidation, condensation, and polymerisation reactions. Both parameters showed a decrease of 16% with respect to the initial value in the LOTR barrel wines and 13% for *α* and 10% for *αα* in the HOTR barrel wines. This was related to the increase of i throughout ageing, which was the formation of polymeric pigments with the participation of anthocyanins (Fig. 2e), especially between 3 and 6 months, with 25% in LOTR barrel wines and 20% in HOTR barrel wines. In the second part of the ageing the LOTR wines showed a higher polymeric pigment formation than the HOTR barrel wines (Fig. 2d). This result coincided with a higher loss of the wine's initial ACY (Fig. 2c) and with the higher HPP formation in the LOTR barrel wines, which was reflected in a higher colour intensity (Fig. 2f). Thus, LOTR barrel wines presented a colour with higher red colour intensity (higher % 520 and dA%). This was significantly higher in LOTR barrel wines than in HOTR ones at 6 months (Table 3). This result was consistent with the higher IoIn observed in LOTR barrel wines. The compounds responsible for the brownish tones (%420) were more important in the colour of the HOTR barrel wines than in the LOTR ones, this difference being significant at 6 months. These results indicated that in the wines from barrels with less OTR there was a greater formation of these new pigments responsible for red and blue tones (Alcalde-Eon, Escribano-Bailón, Santos-Buelga, & Rivas-Gonzalo, 2006; Fernandes et al., 2017), meaning the HOTR barrel wines showed a more evolved colour than those from LOTR barrels.

Colour intensity (CI) remained stable until 6 months of ageing. Thus, in the first 3 months, a slight increase in absorbance was observed in all the wines, caused by compounds responsible for brown (absorbance 420 nm) and blue (absorbance 620 nm) tones, together with a slight decrease in the red colour of the wine (absorbance 520 nm), which increased slightly after 6 months. The slight increase in absorbance at 420 nm has been attributed by some authors to the extraction of compounds from the oak wood (Perez-Prieto et al., 2003) which was an increase of 2% and 1% for the LOTR and HOTR barrels, respectively, reflecting the greater release of compounds from the wood of the LOTR barrels. The 6-month increase in absorbance at 620 nm and 520 nm of the wines may be due to the formation of new compounds by ethyl-bridged polymerisation processes and co-pigmentation phenomena in the wine, which generate violet-red compounds (Chassaing et al., 2010; Fernandes et al., 2017). A higher increase in absorbance at both 520 nm and 620 nm was found in wine from LOTR barrels, namely 5% and 2%, respectively, compared to 3% and 1% in HOTR barrels. Possibly due to the formation of new anthocyanin-derived pigments, anthocyanin-aldehyde-flavanols pigments, such as Mv-3-Gl-Ethyl-Cat and Mv-3-CoumGl-Ethyl-Cat, which have a maximum absorption wavelength around 540 nm and were described in the literature as

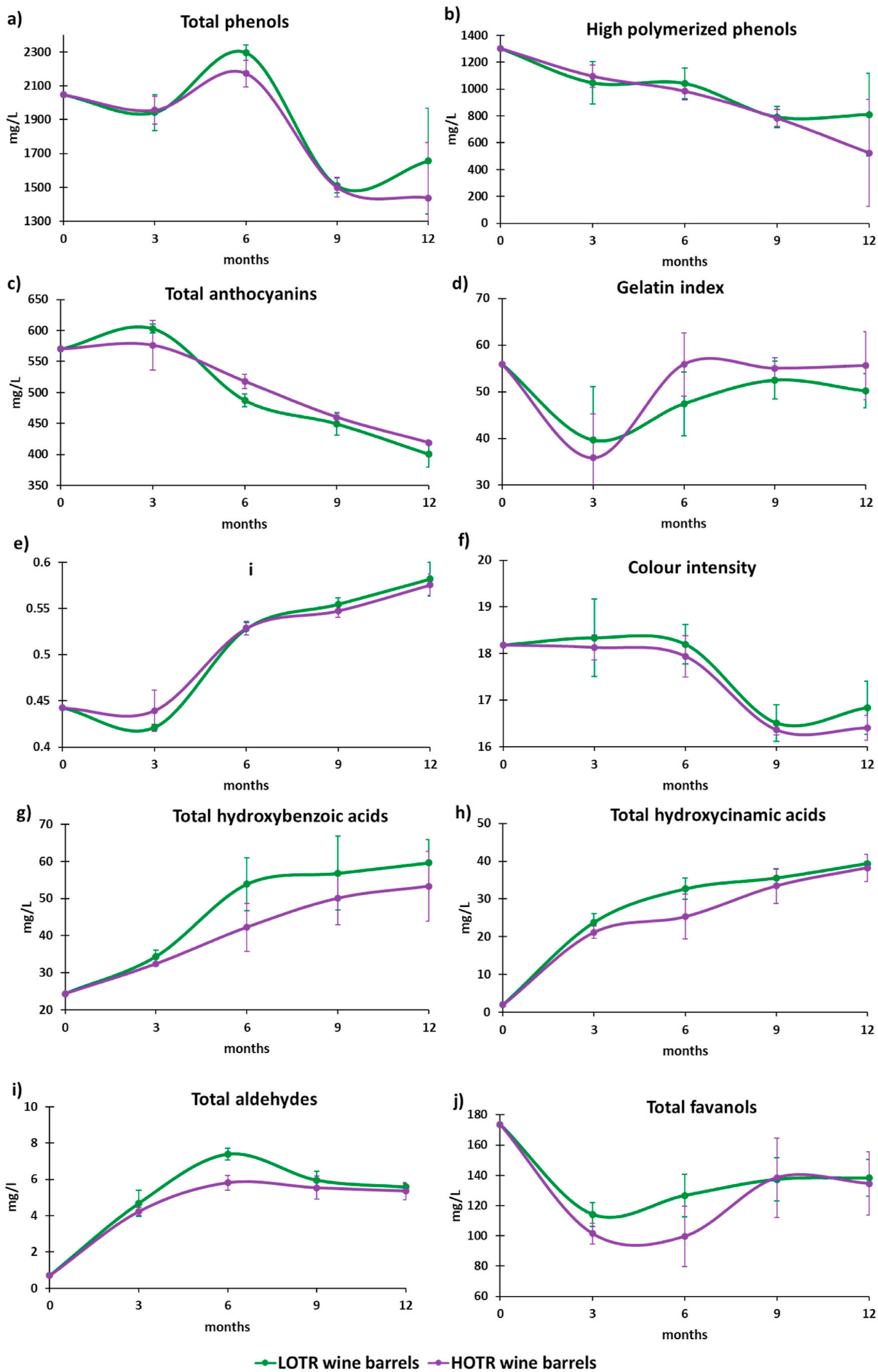


**Fig. 2.** Graphical representation of the evolution of some compounds during the wine ageing in 4 LOTR and 4 HOTR barrels.

contributing to the violet hues (Vidal, Meudec, Cheynier, Skouroumounis, & Hayasaka, 2004). After 9 months of ageing, a significant decrease in CI of 5% and 12% of the absorbance at 420 nm and 520 nm in both types of wines was observed in all the wines studied. In the case of absorbance at 620 nm, higher losses were found in the wines from LOTR (9%) compared to 8% in the wines from HOTR barrels. Therefore, after 12 months of ageing, the wine showed 8% less CI compared to its situation before starting the ageing in LOTR barrels while the decrease was 10% after ageing in HOTR barrels. This result reflected the higher colour stability of the polymeric pigment formed in LOTR barrel wines, while HOTR wines maintained a higher content of the wine's initial anthocyanins which may be involved in oxidation reactions leading to the loss of colour in red wine (Gortzi, Metaxa, Mantanis, & Lalas, 2013).

### 3.4. Low molecular weight phenols (LMWP), catechin and epicatechin

Table 4 presents the comparative content of low molecular weight compounds in wines aged in LOTR and HOTR barrels after 3, 6, 9 and 12 months. The ageing time defined the evolution of these compounds in the wines, as already described in other works which, in addition to time, studied the type of wood or the ageing system (del Alamo-Sanza et al., 2010; del Alamo Sanza, Fernández Escudero et al., 2004; Gallego et al., 2012; Rubio-Bretón et al., 2018). Furthermore, the LMWP composition of the wood that will be in contact with the wines determines the extraction and therefore the content in the final wines (Martínez-Gil et al., 2020). The evolution of the LMWP content was similar in wines aged in LOTR and HOTR barrels. The major compounds were flavanols (catechin and then epicatechin), which decreased during ageing.

The total content of the hydroxybenzoic acids analysed (gallic acid, protocatechuic acid, vanillic acid, syringic acid and gentisic acid) can be seen in Fig. 2h showing a higher content in the wines aged in LOTR barrels than in those from HOTR barrels, with the content of all those acids being significant at 6 months of ageing and also at 3 months in the case of gallic acid. However, no differences were found in the protocatechuic acid content at 9 months but higher levels of gentisic acid were found in the HOTR barrels at 9 and 12 months.

Gallic acid is the compound yielded by the wood and found in the highest concentration in both LOTR and HOTR barrel wines. During barrel ageing, the gallic acid content in the wine increased, possibly due to the hydrolysis of tannins released from the wood, as described by several authors (del Alamo Sanza, Nevares Domínguez, Cárcel Cárcel, & Navas Gracia, 2004; Gallego, Nevares, Fernández, & delAlamo, 2011; Viriot, Scalbert, Hervédu Penhoat, & Moutounet, 1994). It is an important compound due to its strong antioxidant activity even at very low concentrations (Rubio-Bretón et al., 2018). The greatest increase in gallic acid occurred at 6 months, increasing the initial concentration by 80% in wines aged in LOTR barrels and 40% in HOTR barrels. Therefore, as mentioned above, the wines aged in LOTR barrels extracted more compounds from the wood and more quickly, probably because the classification of wood by OTR implies a differentiation of anatomical and therefore compositional characteristics. Thus, the wines in HOTR barrels needed twice as long, 12 months, to reach the gallic acid content of the LOTR barrel wines at 6 months and at the end of ageing the LOTR wines had multiplied the initial concentration of gallic acid by 2.1 (Table 4).

The protocatechuic acid, vanillic acid and syringic acid content increased in the wines more markedly in the first 3 months of ageing, being higher in the wines from ageing in LOTR than in HOTR barrels. Thus, the wines from LOTR barrels at 3 months showed an increase of 138%, 334% and 459% with respect to the initial concentration of protecatechuic acid, vanillic acid and syringic acid, respectively, compared to the increase of 120%, 306%, 455% found in the wines from HOTR barrels. After 6 months of ageing, the differences between HOTR and LOTR barrel wines were more pronounced, with the LOTR ones showing 17%, 14% and 27% higher contents of protocatechuic acid, vanillic acid and syringic acid than those aged in HOTR barrels. The concentration of protecatechuic acid in the wines continued to increase up to 12 months and was recorded 7% higher in the wines from LOTR barrels than from HOTR ones. The syringic acid content during the first 6 months of ageing remained constant in the wine from HOTR and decreased in the wine from LOTR barrels (Table 3). This evolution of these hydroxybenzoic acids (gallic acid, protocatechuic acid, vanillic acid and syringic acid) agrees with the results of other authors and can be explained by the fact that they come from the hydroalcoholysis of oak wood and are transferred to the wine during ageing (del Alamo Sanza, Nevares Domínguez et al., 2004; Rubio-Bretón et al., 2018).

Gentisic acid had an irregular trend over time, with fluctuations in concentration observed throughout the 12 months of ageing. Other authors have described this behaviour, the increase being related to wood release, as it is one of the main phenolic acids in oak (Zhang, Cai, Duan, Reeves, & He, 2015), which would increase its concentration in the wine during ageing. The decrease is due to the fact that it takes part in the oxidative condensation and browning processes during wine ageing (del Alamo Sanza, Nevares Domínguez et al., 2004). Gallego et al. (2011) observed that wines with 5 months of wood showed a large increase of gentisic acid that decreased in bottle until it disappeared after 5 months. Similar results were described in the work of del Alamo Sanza, Nevares Domínguez, et al. (2004) showing a constant decrease of gentisic acid in the wine throughout ageing in wood. In this work the content of this compound was found to show an irregular trend, decreasing more markedly at the beginning of ageing from 0 to 3 months with the wines from HOTR barrels showing 36% less gentisic acid while those from LOTR barrels showed 34% less. However, in the 3–6 month and 9–12 month periods, an increase in gentisic acid was observed, more pronounced in the middle of the ageing period and in the LOTR barrel wines which showed 20% more than the HOTR ones. At the end of ageing, the balance of this irregular trend indicated that after 12 months wines in barrels showed lower content than at the beginning.

The hydroxycinnamic acids studied in the wines were coumaric acid and caffeic acid, those most common in oak (Zhang et al., 2015). As in the case of the hydroxybenzoic acids, the highest concentrations were found in the wines aged in LOTR barrels (Fig. 1h), which were significantly higher than in the HOTR wines at 3 and 6 months of ageing (Table 4). The content of both acids from wood increased continuously in all wines throughout the ageing period (del Alamo Sanza, Fernández Escudero, & De Castro Torío, 2004; delÁlamo Sanza et al., 2004; Gallego et al., 2011). Like most of the hydroxybenzoic acids, the highest increase was observed at 3 months of ageing, in line with other studies. In the first months of barrel ageing, the wine impregnated the wood, advancing up to 4 or 5 mm depending on the type of wine and wood, thus producing the greatest extraction. In this work, the increase in concentration in this period, with respect to the initial concentration of the wine, was 7.6 and 6.1 times for coumaric and 13.4 and 12.2 times for caffeic acid, for the LOTR and HOTR barrel wines, respectively.

After 6 months of ageing, the wines from LOTR barrels contained 31% and 28.6% more *p*-coumaric acid and caffeic acid, respectively, than the wines from HOTR barrels. In the second half of the ageing, the increase was smaller, but of greater significance in the HOTR barrels than in the LOTR ones, so the content was similar in the wines from both types of barrels at the end of ageing. This result coincided with what was described above, the LOTR barrel-aged wines extracted more compounds from the wood and more quickly. The colour of red wines is strongly influenced by the presence of these phenolic acids, as they play an important role in the pigment enhancement and stabilisation of red wines through intra- and intermolecular co-pigmentation reactions, especially hydroxycinnamic acids, which can show a 60%–70% colour enhancement at 520 nm (Zhang et al., 2015). This could explain the increase in absorbance at 520 nm and %520 observed from months 3–6 in the wines from the different barrels (LOTR and HOTR) and was more prominent in those from LOTR barrels (Table 3).

The total aldehyde content was higher in wines from LOTR ageing than in HOTR barrels (Fig. 1i). Both vanillin and *p*-hydroxybenzoic aldehydes were significantly higher in wines aged in LOTR than in HOTR barrels at 6 months. They may also moderately affect the bluish colour of the wines, due to their participation in reactions with anthocyanin (Gallego et al., 2011). *p*-hydroxybenzoic increased the initial wine concentration 4-fold in wines aged in LOTR barrels and 3.3-fold in HOTR barrels in the first 6 months. Vanillin is the most important low molecular weight compound from an organoleptic point of view, contributing directly to the aroma of the wine with sweet and vanilla notes, provided it is found in concentrations above its perception threshold (60 μg/l) (Culleré, Escudero, Cacho, & Ferreira, 2004). In this case, the initial wine increased its concentration 13.22 times in the first half of ageing in LOTR barrels, and 11.22 times in HOTR barrels. Therefore, at 6 months wines from LOTR barrels had a 28% higher vanillin concentration than wines from HOTR barrels, probably due to the higher vanillin content in LOTR than in HOTR wood (Sánchez-Gómez, del Alamo-Sanza, & Nevares, 2020). However, after 9 months, a decrease in the concentration of this aldehyde in the wines was observed, higher in the LOTR than in the HOTR barrels, and although the LOTR wines still had a higher concentration at 12 months (3.5% more), they were no longer significantly different.

In general, the concentration in the wines of the main wine flavanols (catechin and epicatechin) decreased during ageing, remaining at higher levels in the LOTR than in the HOTR barrel wines (Fig. 1j), the difference being significant at 3 and 6 months of ageing (Table 4). This greater decrease occurred in the first 3 months and was more pronounced in HOTR than in LOTR barrel wines, with 35% for catechin and 55% for epicatechin in HOTR compared to 27% and 49%, respectively, in LOTR barrel wines. These results agree with those observed by other authors (Cano-López, Pardo-Minguez, López-Roca, & Gómez-Plaza, 2006; delÁlamo Sanza et al., 2004; Gallego et al., 2011; Rubio-Bretón et al., 2018). The loss of these compounds may be due to their participation in the processes of polymerisation and condensation with anthocyanins, reactions favoured by the continuous diffusion of oxygen from the barrels and their adsorption on the surface of the wood, while the slight increases detected may be due to the hydrolysis of higher oligomers (Rubio-Bretón et al., 2018). Therefore, the wines aged in LOTR barrels maintained the concentration of flavanols of the initial wine better because they participated in fewer reactions than those aged in HOTR barrels. This may be due to the fact that the higher oxygen supply in wines from HOTR barrels favours the interactions of wine and wood compounds leading to a greater decrease of these flavanols.

### 3.5. Differentiation of aged wines

For an overall view of the influence that the OTR of the barrels has on the characteristics of the wines during the ageing year, a principal component analysis was carried out to evaluate the differentiation of the wines in each month of sampling. The analysis used the data of the parameters that showed statistically significant differences in the wines aged in HOTR and LOTR barrels (Tables 1, 3 and 4) for each ageing time. The graphical representation of the representative samples of each barrel, in the space defined by the two main functions obtained, shows a distribution that allows all the wines aged in high and low OTR barrels to be clearly distinguished.

Fig. 3a shows that after 3 months of ageing, the first principal component, which accounts for 54%, distinguishes between wines aged in HOTR and LOTR barrels. Thus, the wines from the 4 HOTR barrels are located on the positive axis of PCA1, showing the highest α, TA and % 420, while the wines from the LOTR barrels are defined by a higher content of low molecular weight compounds, Cu and C–$SO_2$ (Fig. 3b). These results highlight what was described above: on the one hand, that indeed the same wine evolved differently in barrels with different oxygenation rates and, on the other hand, that LOTR barrels transferred a higher content of phenolic compounds from the wood to the ageing wines, which would indicate a higher wine-wood interaction. This scenario was maintained after 6 months of barrel ageing (Fig. 3c). In this case the first principal component explains 65% of the variability of the wines and is positively correlated with %420, ACY, TAN, LPP, EtIn which is where the wines from HOTR barrels are located (Fig. 3d). However, the LOTR wines located on the negative axis of PC1 are defined by low molecular weight phenolic compounds, catechin, epicatechin, HPP, αα and %520. This indicates that these wines underwent more polymerisation processes with the formation of red compounds and presented higher levels of ethyl acetate, which could be related to a greater extraction of acetyl groups from the wood as precursors of this compound. At 9 months of ageing, PCA1 explains almost 66% of the variability and allows the differentiation of wines from HOTR and LOTR barrels (Fig. 3e). Thus, low molecular weight phenolic compounds were found to be less important in LOTR barrel wines. HOTR wines are on the negative side of the first main component defined by the major volatile compounds, LPP, G+F, and are defined by the loss of free $SO_2$, which leads to an increase of the combined sulphur dioxide C–$SO_2$ (Fig. 3f), while in LOTR barrel-aged wines, Cu and IoIn are more important. The importance of the copper content in the wines, which increases during ageing, is related to the transfer from the wood, as was also observed after 3 months of ageing. At the end of the study, the wines from the HOTR and LOTR barrels differed according to the PCA1 which explains 77%, mainly with the variables C– $SO_2$, TA in the case of the wines from the HOTR barrels and with methanol, Cu and pH in the case of the wines from the LOTR barrels. The wines from the 4 HOTR barrels showed greater homogeneity with ageing time than those from the LOTR barrels.

## 4. Conclusions

The level of oxygen transfer from barrels made of *Quercus petraea* (Matt.), Liebl. wood from the same batch classified by their OTR determined the final composition and colour of the aged red wine. The basic parameters of the wines aged in high or low oxygenation barrels were similar during the 12 months of ageing although the rest of the parameters and compounds analysed differed. The release of compounds and the reactions that occurred during ageing were faster in the case of the wine aged in low oxygenation barrels (LOTR), while those in HOTR barrels underwent these processes more gradually throughout the ageing. These differences were more relevant at 6 months but less significant with ageing time.

Wines aged in LOTR barrels showed lower anthocyanin and tannin content due to the development of more condensation and polymerisation reactions, reflected in a higher content of highly polymerized phenols and polymeric pigments. On the other hand, wines aged in HOTR barrels retained more anthocyanins and tannins showing more tannic character. This was reflected in the colour of the wines, as the wines aged in LOTR barrels showed greater colour intensity throughout the ageing period.

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## Conflict of interest and authorship conformation form

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○ All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.

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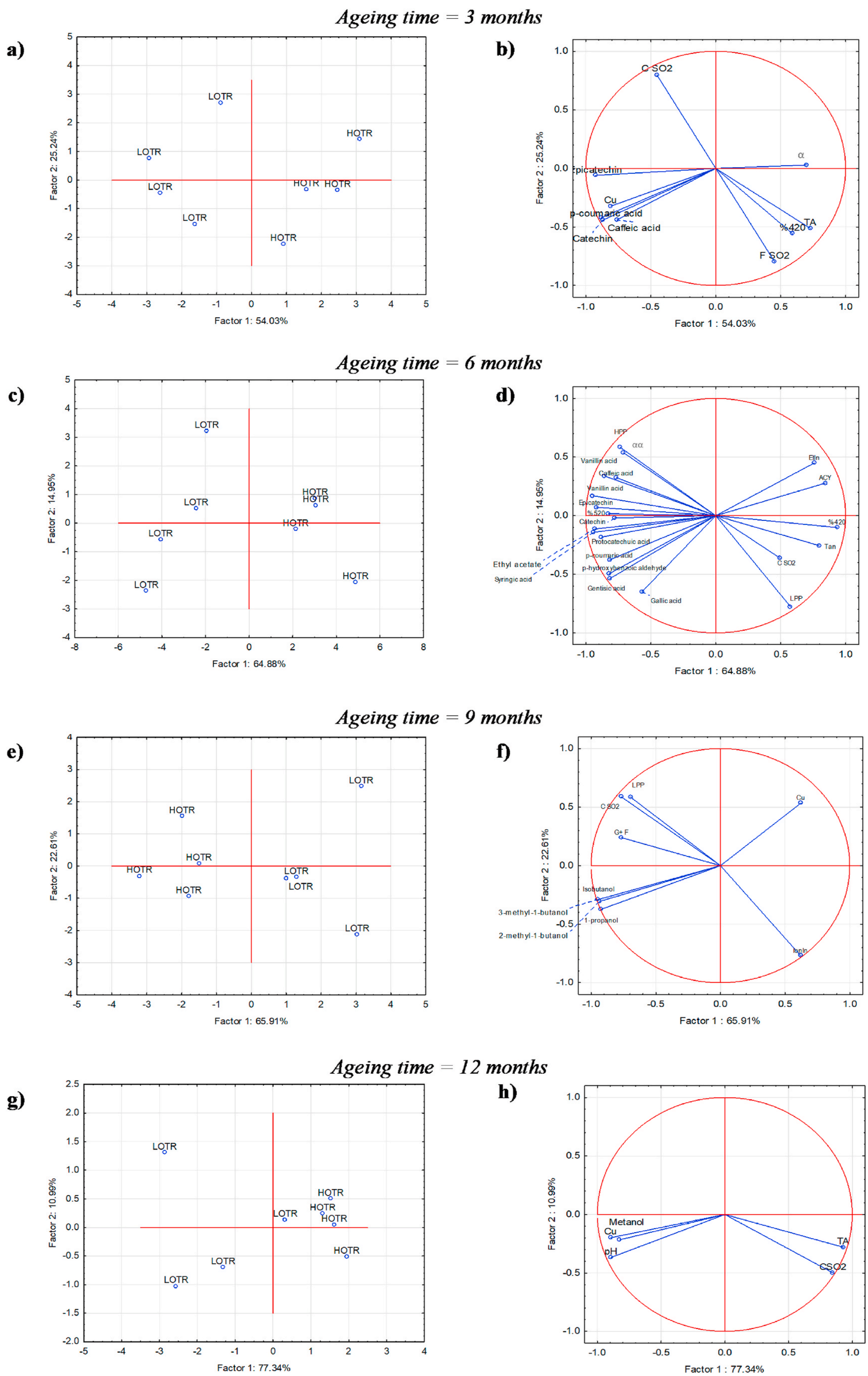


**Fig. 3.** Principal Component Analysis (PCA) performed with the data of the parameters that showed statistically significant differences in the wines aged in 4 HOTR and 4 LOTR barrels for 3, 6, 9 and 12 months.

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## CRediT authorship contribution statement

**Ana Martínez-Gil:** Writing – review & editing. **Maria Del Alamo-Sanza:** Conceptualization, Methodology, Formal analysis, Supervision, Project administration, Funding acquisition. **Ignacio Nevares:** Conceptualization, Methodology, Formal analysis, Supervision, Project administration, Funding acquisition.

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