# Volatile composition of oak wood from different customised oxygenation wine barrels: effect on red wine

Rosario Sánchez-Gómez[a], Maria del Alamo-Sanza[a]*, Ignacio Nevares[b]*
[a] Department of Analytical Chemistry, [b] Department of Agricultural and Forestry Engineering,
UVaMOX-Group, Universidad de Valladolid, 34001 Palencia, Spain
*maria.alamo.sanza@uva.es; ignacio.nevares@uva.es

## Abstract

The compounds that the wood releases to the wine and the oxygen transmission rate (OTR) of the barrel define the final wine. The new possibility of choosing the OTR of the barrel allows the winemaker to globally control the ageing process. The aim of this work was to study the volatile composition of woods classified according to their OTR, which are used to build barrels for wine ageing. The results showed that volatile composition differs depending on wood OTR and the temperature reached during toasting. On the toasted side of the stave in contact with the wine, low OTR wood had a statistically higher content in furan compounds (5-hydroxymethylfurfural, furfural and 5-methylfurfural), acetovanillone and phenolic aldehydes (vanillin and syringaldehyde), while 4-ethylguaiacol and *trans*-*β*-methyl-*γ*-octalactone were significantly higher in staves with a high OTR. The same red wine aged first for three months in high and low oxygenation barrels presents different characteristics.

**Keywords:** *oxygen transmission rate, staves, toasting, volatile compounds, red wine*

## 1. Introduction

The oak wood used to build barrels to age wines and spirits is classified according to its origin and grain type (growth ring width, $L_c$) (Feuillat, Huber, & Keller, 1992; Vivas, 1995). French oak classification is related to forest origin: very wide grain (Large type, $L_c > 5$ mm), wide grain (Limousin type, $4 \leq L_c \leq 5$ mm), average grain (Vosges type, $2 \leq L_c \leq 4$ mm), tight grain (Centre France type, $1 \leq L_c \leq 2$ mm) and very tight grain (Allier type, $Lc < 1$ mm), although others only distinguish open grain > 3 mm and tight grain < 3 mm (Guillaume de Pracomtal, Teissier du Cros, & Monteau, 2014). Vivas (1995) described the existence of a relationship between grain and wood quality, stating that the larger grain woods were richer in extractable compounds and ellagitannins and poorer in aromatic compounds such as eugenol and the *β*-methyl-*γ*-octalactone isomers. Thus, the choice of grain size is related to the volatile and phenolic composition of the oak used to age wines and spirits.

Some studies were later carried out into the relationship between grain size and the volatile composition of the stave wood. Bosso et al. (2008) observed that the grain type of the barrel staves only affected the concentration of *cis*-*β*-methyl-*γ*-octalactone in the wines aged. However, Collins, Miles, Boulton, & Ebeler, (2015) demonstrated that after the cooperage toasting process there were no differences in the volatile composition of the wood previously classified according to its grain size.

The wood used to build barrels goes through a series of cooperage procedures which affect its oenological quality, especially seasoning and toasting (Bosso et al., 2008; Fernández de Simón, Cadahía, del Álamo, & Nevares, 2010). The seasoning stage reduces the wood's initial humidity and induces a series of reactions in the presence of water and sunlight, such as the loss of hydrosoluble phenolic substances (ellagitannins). These are directly related to decreased bitterness and astringency while at the same time increasing certain aromatic compounds. Natural seasoning in the open air, considered the most widely used method in cooperage, affects the different aromatic compounds of the wood (lactones, phenolic aldehydes or volatile phenols, among others) in varying ways. Some increase their concentration while others decrease it or do not show any significant variation before and after seasoning. The toasting stage is considered to affect the chemical composition of the wood most and particularly its volatile composition (Doussot, De Jéso, Quideau, & Pardon, 2002). This process produces various modifications in the volatile composition as a result of thermodegradation of the cellulose, hemicelluloses and lignin. When these macromolecules of the wood decompose, new volatile compounds can be generated (Cadahía, Fernández de Simón, & Jalocha, 2003) and the content of some of them can be increased (Fernández de Simón, Esteruelas, Muñoz, Cadahia, & Sanz, 2009) or that of others decreased (Bosso et al., 2008). It needs to be stated that several studies have brought to light contradictory results regarding the impact of toasting temperature on the generation of volatile compounds in oak: some report an increase and others a decrease in the same substances with an increased toasting temperature (Duval et al., 2013). In any case, both seasoning and toasting will affect the final chemical composition of the barrel stave wood.

In the last few years, and in order to classify wood for building barrels according to ellagitannin content, an on-line system to estimate total phenol concentration, mainly ellagitannin, has been developed (Oakscan, Radoux, France). This classification system arose in order to decrease variability in the content of these compounds in the wood selected for building barrels, since that related to wood grain resulted in a significant variation in ellagitannin and polyphenol levels. It is a non-destructive measuring method which uses near infra-red spectroscopy (NIRS) to classify the wood (Michel et al., 2013). The staves are classified in three categories according to the Total Polyphenol Index (IP) and these are directly related to ellagitannin content (Michel et al., 2016).

In addition to contributing wood compounds to the wine, the barrel acts as an active container which doses oxygen to the ageing wine at the same time as a series of phenomena conditioning its performance occur, such as the formation of head space, the loss of wine due to the wetting of the wood and evaporation or the generation of an interior depression, among others (del Alamo-Sanza & Nevares, 2017). All this directly or indirectly determines a variety of phenomena suffered by the wine during the ageing process (polymerization of tannins and anthocyanins, consumption of free sulphur dioxide, oxidation of ethanol and acetaldehyde, general modifications in the wine's phenolic profile among others), thus determining its final characteristics (Nevares & del Alamo-Sanza, 2015). These phenomena reflect changes in the wine's compounds as well as its interactions with those extracted from the wood, hence defining the final wine. The parameter indicating the quantity of oxygen passing to the wine via the barrel is called the oxygen transmission rate (OTR) and reflects its velocity on entering the barrel through the joints between staves, the stopper and the wood itself (Vivas & Glories, 1997) and, for a specified time, defines the total quantity of oxygen passed to the wine. The OTR of the wood used to build barrels depends, among other aspects, on its species, geographical origin, density and anatomical properties (del Alamo-Sanza, Cárcel, & Nevares, 2017). In the case of French oak (*Quercus petraea* Liebl.) the quantity of oxygen entering the wood can contribute up to 75 % of all that received from a new barrel (Nevares Domínguez & del Alamo-Sanza, 2014) so, in this case, the type of wood determines both the compounds and the quantity of oxygen contributed to the wine during the process.

Given that the OTR of the wood is related to its anatomical properties (Nevares et al., 2019) , the aim of this study was to determine the relationship between the OTR and volatile composition of oak wood and, on the other hand and for the first time, to evaluate the ageing of the same red wine during 3 months in oak barrels made with *Quercus petraea* Liebl. wood by the same cooperage and classified as having a low or high oxygen transmission rate.

## 2. Materials and Methods

### *2.1. Chemicals and reagents*

Methanol (supragradient HPLC grade) and dichloromethane (HPLC grade, stabilised with ethanol) were purchased from Scharlab, S.L. (Barcelona, Spain). Ethanol absolute AGR ACS ISO was purchased from Labbox Labware, S.L. (Barcelona, Spain). 3 mL prepacked cartridges filled with 200 mg of LiChrolut EN resin were purchased from Merck (Darmstadt, Germany). Compressed air, nitrogen and hydrogen with GC grade were purchased from Carburos Metálicos (Barcelona, Spain). Volatile standards (2-octanol, furfural, 5-methylfurfural, $\gamma$-butyrolactone, guaiacol, *trans*-$\beta$-methyl-$\gamma$-octalactone, *cis*-$\beta$-methyl-$\gamma$-octalactone, 4-methylguaiacol, 4-ethylguaiacol, 4-ethylphenol, eugenol, 4-vinylguaiacol, isoeugenol, 5-hydroxymethylfurfural, ethyl vanillate, vanillin, acetovanillone and syringaldehyde) were purchased from Sigma Aldrich (Steinheim, Germany). Water was purified using a Milli-Q system (Millipore, Bedford, USA).

### *2.2. Wood and barrels*

Three thousand fresh staves from *Quercus petraea* Liebl. oak wood, slightly longer than usual, seasoned in the open air for three years in the wood yard at cooperage in the usual way (INTONA, Navarre, Spain), were classified by their OTR based on the anatomical features of the wood by means of image analysis and artificial neuronal network (Martínez-Martínez, del Alamo-Sanza, & Nevares, 2019). Two well-differentiated groups of staves classified as high and low OTR were obtained. From these two groups, ten staves, as homogeneous as possible, with a low OTR (L-OTR) and another ten with a high OTR (H-OTR) were selected with the aim of having two completely differentiated groups with very different OTR values. Once the heads of these twenty staves had been cut for analysis (henceforth Samples A), the remaining parts of each selected fresh stave were sent to the cooperage for the construction of a barrel. At the same time, with the rest of the staves from each group, homogeneous batches were prepared for the construction of eight barrels of each type (L-OTR and H-OTR). A total of seventeen barrels were built in the cooperage following the usual process of bending and toasting, according to the cooperage protocol, to a medium degree by radiation. Barrels were heated with a series of wood fires of medium intensity from room temperature up to 180 ºC and maintained at this temperature for 40 minutes, 20 minutes on each side. Prior to that, barrels were submitted to a 30 minutes 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 ºC. The temperature in both cases (inside and outside) was measured by an infrared thermometer. The built barrel including the ten L-OTR staves as well as the ten H-OTR staves was disassembled, and the twenty staves were sent to our centre for analysis. Half of the remaining barrels (four L-OTR barrels and four H-OTR barrels) were used to evaluate the actual OTR (Prat-García, Nevares, Martínez-Martínez, & del Alamo-Sanza, 2020), and the other half (four L-OTR and four H-OTR barrels) were used to age a red wine.

### *2.3. Wood oxygen transmission rates (OTR) and wood characteristics*

Table 1 presents the wood properties area of earlywood (EW), latewood (LW), density and grain size (G) of the twenty fresh staves obtained by image analysis according to Martínez-Martínez et al. (2019). The real OTR was measured according to del Alamo-Sanza & Nevares (2012) and the group of low OTR staves had an average OTR between 0.0003 to 0.0082 mg/L·day while an average OTR between 0.054 to 0.092 mg/L·day was obtained in the high OTR staves.

### *2.4. Red wine*

A young red wine (Cabernet Sauvignon) produced in 2017 in the region Ribera del Duero (Castile and León, Spain) was used. The initial chemical parameters of the wine were evaluated according to OIV (International Organization of Vine and Wine) methods (OIV, 2003): total acidity 4.56 g/L, expressed as tartaric acid; volatile acidity 0.48 g/L, expressed as acetic acid; sugars 1.4 g/L; alcohol strength 15.16 %; colour intensity 15; and total polyphenol index 61. The wine was transferred into the eight barrels (225 L) and samples were collected from each of the barrels after three months of ageing. The barrels were stored in the same ageing room in the experimental winery at the University of Valladolid (Palencia, Spain), where humidity and temperature conditions were controlled at 65–75 % and 15–16 ºC, respectively.

### *2.5. Extraction of wood volatile compounds*

Two types of samples were obtained from the toasted staves: a) the outer layer of the staves (practically untoasted) cut to a depth of 17 mm with a brush-rusher Holzstar AHD 200 (Stürmer Maschinen GmbH, Hallstadt, Germany), which represent the part of the stave not flooded by wine (from now on Samples B); and b) the inner layer of the stave (toasted) with a depth of 10 mm (from now on Samples C), which represent the maximum thickness flooded by wines or spirits during the ageing process (Figure 1). Samples B reported the average concentration of the compounds analysed which could be found in the thickness of the stave not flooded with wine and which reached a temperature between 60 ºC and 135 ºC during toasting. Samples C provided more exact information on the part of the stave which, presumably, would be in contact with the wine during the ageing process in barrel and which had been in direct contact with the barrel toasting brazier reaching temperatures between 135 ºC and 180 ºC. Figure 1 summarizes the toasting scheme and the samples collected from the staves. A total of 60 samples were analysed from fresh staves (samples A) and from toasted staves (samples B and samples C).

The pieces of wood were ground and sieved using an ultracentrifugal mill Retsch ZM 200 (Retsch GmbH, Haan, Germany), obtaining sawdust of a particle size less than 1 mm. All samples were stored until extraction under environmental conditions of 16 °C temperature and 75 % relative humidity.

The sawdust from each stave (10 g) was mixed with 22 mL of a methanol/water solution (15:85, v/v). Extraction was carried out with an Elma Ultrasonic bath T700/H (Elma GmbH & Co KG Kolpingstr, Singen/Germany) for 15 minutes. All extracts were centrifuged at 4000 rpm for 10 min at 0 ºC and the supernatant was separated. Subsequently, the solid sample was extracted another three times until exhaustion using the same volume of the methanol solution (22 mL). The four consecutive supernadants were mixed. All extractions were carried out in duplicate for each stave sample. After wood extraction, 50 mL of each extract was taken and 200 µL of an internal standard (2-octanol, in a dose of 5 g/L in ethanol) were added.

The mixture of wood extract and standard was then extracted following the methodology described by Lopez, Aznar, Cacho, & Ferreira (2002) for which prepacked cartridges (total volume 3 mL) were used. For this, the 50.2 mL of the mixture were passed through the SPE cartridges at 2 mL/min. Subsequently, the sorbent was dried by letting air pass through it at approximately −0.6 bar for 30 min. The resulting analytes were recovered by elution with 1.3 mL of dichloromethane.

### *2.6. Extraction of wood volatile compounds from red wine*

Volatile compounds from the wine were extracted according using the Lopez et al. (2002) method. Briefly, 50 mL of centrifuged wine (under the same conditions as the extracts) with 200 µL of an internal standard (see previous section) were passed through the prepacked cartridges under the conditions indicated above.

### *2.7. GC-FID analysis*

The separation, identification and quantification of volatile compounds from the wood extracts and the wines were carried out using a Hewlett-Packard 6890 gas chromatograph (Palo Alto, CA, USA) equipped with a flame ionisation detector (FID). Samples were injected in split mode (1:10, 0.1 mL/min) and volatiles were separated using a capillary column (HP-Innowax) (30 m x 0.25 mm i.d., and 0.25 µm film thickness) with GC grade nitrogen as a carrier gas at a flow rate of 1 mL/min. The working conditions were the same as those used by Fernández De Simón, Cadahía, & Jalocha, (2003), with some modifications: injector temperature, 250 ºC; detector temperature, 250 °C; column temperature, 45 °C, heated at 3 °C/min to 230 °C, held for 25 min, and then heated at 10 °C/min to 250 °C (held for 10 min) (del Alamo-Sanza, Laurie, & Nevares, 2015).

In all cases, the samples were injected twice in GC-FID. The compounds were identified by comparing their retention times with those of the pure reference standards. The compounds evaluated were: furfural, 5-methylfurfural, $\gamma$-butyrolactone, guaiacol, *trans*-$\beta$-methyl-$\gamma$-octalactone, *cis*-$\beta$-methyl-$\gamma$-octalactone, 4-methylguaiacol, 4-ethylguaiacol, 4-ethylphenol, eugenol, 4-vinylguaiacol, isoeugenol, 5-hydroxymethylfurfural, ethyl vanillate, vanillin, acetovanillone and syringaldehyde. The concentrations of each substance were measured by comparison with standard calibration curves made in a methanol/water solution (15:85, v/v) for wood and in model wine (12% ethanol, 3.5 g of tartaric acid and pH=3.6) for wines, with pure reference compounds analysed under the same conditions. The corresponding calibration was performed for each compound and each matrix with seven points and linear regression coefficients between 0.98 and 0.99 were obtained. In all cases, the samples were analysed in duplicate.

### *2.8. Statistical analysis*

The existence of statistically significant differences in the concentration of volatiles in each high and low oxygenation wood sample were determined using one-way analysis of variance (ANOVA) at the 95% probability level according to Fisher's least significant difference (LSD). Discriminant analysis was used as the method for multigroup discrimination to study the capacity of the data corresponding to the concentration of volatile compounds in order to classify the wood samples and wines in predefined groups. In order to compare the data for wood and wine, it was necessary to carry out a standardisation process. For this purpose, the variables were transformed into reduced centre variables, thus ensuring that the correlation coefficients between reduced centre variables remain identical to those before standardisation. The dataset used consisted of the level of volatile compounds in the wood determined in the various samples taken from both the high and low oxygenation staves and wines aged in high and low OTR barrels for 3 months. The statistical analysis was performed using the Statistica program (version 64; StatSoft, Inc., USA).

## 3. Results

### *3.1. Characteristics of the oak woods studied*

The features of the fresh staves chosen according to their OTR prior to toasting are shown in Table 1, detailing the median characteristics for each group. The fresh H-OTR staves had a mean value of 0.075 ± 0.012 mg/L·day for this parameter in contrast to the 0.004 ± 0.003 mg/L·day recorded in the L-OTR staves. The mean oak wood density value of all the fresh staves (L-OTR and H-OTR) was 0.681 g/cm$^3$, with extreme values for both groups between 0.479 g/cm$^3$ and 0.815 g/cm$^3$ (data not shown). Wood density and the other anatomical features of the wood, such as earlywood (EW) and latewood (LW) percentage as well as the grain size (G), were not statistically different in the two groups studied.

### *3.2. Study of the effect of toasting temperature on the compounds in high and low oxygenation wood*

The samples (A, B and C) from each group of staves (H-OTR and L-OTR) were described in accordance with their volatile composition (Figure 2). The comparison between the results of samples

A (corresponding to the fresh untoasted wood) and samples C (corresponding to the innermost layer of the toasted staves (10 mm), which is the maximum thickness in contact with the wine or spirit during ageing) is shown in Table 2.

Once the content of each compound studied in the staves was known, the effect of temperature on the variation in volatile compound content was analysed in the 27 mm stave thickness of high and low oxygenation woods (Figure 2). The average content found in each zone analysed was represented in the stave thickness. The 27 mm point corresponded to the untoasted wood (samples A); the 18.5 mm one represented the average found in the thickness of the toasted stave which does not come in contact with the ageing wine (samples B, from the outer part of the toasted stave) and the 5 mm one showed the average content of the toasted wood with a thickness of 10 mm, which is flooded by the wine (samples C, from the inner part of the toasted stave) (Figure 2A). This information revealed the content variation of the compounds generated during the toasting process in the high and low oxygenation woods and which could be extracted by the wine according to its greater or lesser infiltration into the wood throughout the ageing process.

The temperatures reached during toasting throughout the 27 mm thickness of the staves and the degree of concentration in the wood are also shown in Figure 2, with the mean values for each compound studied for each group of L-OTR and H-OTR staves. It was considered that the inner surface was subjected to 180 ºC and the outer stave surface to 60 ºC, assuming a uniform thermal conductivity in the thickness of the staves, to calculate the temperature reached in each zone. Bearing in mind that heat penetrates the wood slowly in the toasting process (due to its low thermal conductivity), thermal degradation is gradual and limited in depth, meaning the quantity and nature of the potentially extractable substances can be different. Due to the fact that the highest temperature (180 ºC) was reached in the inner surface of the staves (samples C) it was important to emphasize that the concentration levels found in these samples reflected the combined effect of two processes: on one hand, the generation of volatile compounds emerging from the precursors present in lignin, cellulose and hemicelluloses and, on the other, the disappearance of some of these on reaching temperatures causing their degradation (Farrell et al., 2015). Quantification of each compound studied reflected this balance with a greater or lower concentration in the different cases.

The three first compounds studied were furanic (5-hydroxymethylfurfural, furfural, and 5-methylfurfural), and no differences were found in their content between the two groups of staves in the case of samples A (Table 2 and Figures 2B, 2C and 2D). 5-Hydroxymethylfurfural presented a higher concentration in the L-OTR woods with a value of 10.64 µg/g. The effect of toasting on these compounds produced a significant increase in their concentration in both L-OTR and H-OTR staves, reaching their highest values on the inner surface of the staves (5 mm point, Samples C) (Figures 2B, 2C and 2D): the mean values found for 5-hydroxymethylfurfural, furfural, and 5-methylfurfural were approximately 25, 14, and 2.2 µg/g for L-OTR staves and 19, 8 and 1.5 µg/g for H-OTR staves. A higher content of the three compounds was quantified in the L-OTR samples (Figures 2B, 2C and 2D), in spite of the fact that only the 5-hydroxymethylfurfural (Figure 2B) concentration in untoasted surface samples (thickness = 27 mm) was slightly higher than in the H-OTR ones. Concentrations were lower than the previous ones in the central stave zone (~18.5 mm), where the mean temperature reached during toasting was approximately 97.5 ºC: 16, 5 and 0.60 µg/g for the L-OTR staves and 9, 3 and 0.5 µg/g for the H-OTR ones for 5-hydroxymethylfurfural (Figure 2B), furfural (Figure 2C) and 5-methylfurfural (Figure 2D), respectively.

No significant differences were observed among syringaldehyde (Figure 2E) and vanillin (Figure 2F) in the two groups of staves in the untoasted samples (samples A) (Table 2). In the L-OTR staves, both compounds were significantly affected by the toasting process with those samples taken from the most toasted zone (samples C) presenting the highest content for both compounds: 9 and 8.5 µg/g, respectively (Figures 2E and 2F). In the case of H-OTR staves, the concentration of both compounds was lower in samples C (6.7 and 7 µg/g) than in the outer thickness of toasted staves (Samples B): 7.4 and 7.4 µg/g (Table 2 and Figures 2E and 2F).

In relation to the volatile phenols, the highest concentrations of both eugenol and isoeugenol were found in the outer part of the stave (samples B, ~18.5 mm): 0.7 and 0.24 µg/g in L-OTR staves and 0.83 and 0.26 µg/g in H-OTR ones for eugenol and isoeugenol, respectively (Figures 2G and 2H). No differences were found in their content between the two groups of staves before and after toasting, A and C samples (Table 2). For the other volatile phenols, such as guaiacol and its derivates, the content of the former was only significantly higher for L-OTR staves in samples C (0.53 µg/g) compared to the untoasted ones (samples A) (0.52 µg/g) (Table 2), but both values were lower than that observed in samples B (0.55 µg/g) (Figure 2I). The highest concentrations of 4-methylguaiacol were quantified in samples C (Figure 2J), with the same mean value for both groups of staves (0.29 µg/g) and a significant reduction in the concentration of 4-vinylguaiacol was recorded in the most toasted samples C (0.635 µg/g) in the H-OTR staves in comparison with samples B (0.645 µg/g) (Figure 2K). However, no differences were found in its content in samples A and C between the L-OTR and H-OTR stave groups (Table 2).

In the untoasted samples (samples A), the two $\beta$-methyl-$\gamma$-octalactone isomers had high relative standard deviation values of between 81 % and 137 % (Figures 2L and 2M). As in the case of previous samples, those from the inner part of the stave (samples C) showed great variability in the concentration of both isomers with value intervals from 0.33 to 1.52 µg/g and 0.33 to 2.85 µg/g for the *trans* isomer and from 0.28 to 13.71 µg/g and 0.28 to 8.97 µg/g for the *cis* isomer in the L-OTR and H-OTR staves, respectively. As a result, the standard deviation varied from 0.47 to 4.83 µg/g for all the staves studied. Figures 2L and 2M shows that the highest concentrations in the L-OTR staves were recorded in the samples from the outer thickness of toasted staves (samples B) with temperatures not above 135 ºC during toasting: 1.1 and 8 µg/g for *trans-β*-methyl-*γ*-octalactone and *cis-β*-methyl-*γ*-octalactone, respectively. Samples from the untoasted staves (samples A) had a lower concentration than the previous ones with 0.85 and 5.5 µg/g for *trans-β*-methyl-*γ*-octalactone and *cis-β*-methyl-*γ*-octalactone, respectively. In the inner part of the staves (samples C), where the mean temperature reached was 157.5 ºC, a decrease in concentration was found in comparison with samples B and A: 0.7 and 3.7 µg/g for *trans* and *cis-β*-methyl-*γ*-octalactone, respectively. These last values were even lower than those quantified in the untoasted staves (samples A). In contrast, the profile of the concentrations in the H-OTR staves increased with the toasting temperature from 1.4 and 4 µg/g for *trans-β*-methyl-*γ*-octalactone and *cis-β*-methyl-*γ*-octalactone, respectively, in the untoasted samples to the higher ones quantified in the inner surface of the stave (samples C), 2.2 and 6.5 µg/g, where the mean temperature reached 157.5 ºC approximately. *trans-β*-Methyl-*γ*-octalactone presented significant differences in samples A and C among the L-OTR and H-OTR staves (Table 2). The third lactone studied was *γ*-butyrolactone (Figure 2N) with different concentration gradients for the two OTR groups, depending on the temperatures reached: it decreased slightly in the L-OTR staves but reached a higher content in the outer thickness of the toasted samples (B) of the H-OTR ones, where the maximum temperature reached was 135 ºC, and decreased in samples C with a maximum temperature of 180 ºC. Thus, the mean concentration in samples C was significantly lower than in the untoasted ones: around 1.6 µg/g in the former in contrast to 1.7 µg/g in the latter (Table 2, Figure 2N).

### *3.3. Multivariate woods and wine analysis*

A discriminant analysis was carried out between the three wood zones of the staves of both OTR levels and wines aged for three months in barrels with low and high oxygenation rates. As a result, two discriminant functions were constructed using sixteen variables (Table 3). Analysis of the coefficients of each variable allowed the significance of each factor to be evaluated. The results are shown in Figure 3 where Factor 1 explained 44.60 % of the variance and Factor 2 explained 36.18 % of the variance.

## **4. Discussion**

### *4.1. Anatomical characteristics of high and low OTR oak woods*

The anatomical features in the analysed staves (Table 1) play a relevant role on the oxygen transmission rate, but according to Nevares et al. (2019) individually they are not sufficient by themselves to directly explain the differences in the OTR of the wood. Significant differences were observed in the OTR of the two groups of staves (L-OTR and H-OTR), but there were no significant differences in the EW and LW areas, nor in grain or density. However, in previous work (Martínez-Martínez et al., 2019) an algorithm was proposed which, based on seven anatomical characteristics of the wood, makes it possible to predict OTR. Therefore all parts of the wood structure together with their corresponding composition define their OTR, meaning that small differences in the structure of the wood could affect its characteristics after the cooperage processes. It is known that EW has a lower density in comparison with LW and EW has vessels of a greater diameter and thinner cell walls in contrast to LW. In addition, EW is more sensitive to thermal degradation than LW (Hamada et al., 2016). In general, less dense woods usually permit more oxygen to pass through, offering a higher OTR value (del Alamo-Sanza et al., 2017), as can also be observed in the mean values in Table 1.

The grain measurement of the fresh woods in both OTR groups (Table 1), indicated that they were tight-grain in accordance with the classification established by (Vivas, 1995) for the French wood used in cooperage. Despite no statistically significant differences being observed between the L-OTR and H-OTR woods for average grain size (2.14 and 1.54, respectively), it should be noted that the larger the grain, the lower the OTR (Nevares et al., 2019). The slight differences in EW and LW values between the woods of the two groups of staves follow the trend described in the bibliography (Vivas, 1995), where lower density woods are described as those with a greater proportion of EW and a lower one of LW.

## *4.2. Effect of toasting temperature on the compounds in high and low OTR oak wood*

The toasting process is known to modify the chemical composition of wood and that the volatile compounds produced by thermal degradation of its main components (polysaccharides such as cellulose and hemicelullose, lignin and lipids) affect the sensorial quality of the wines or spirits aged in the barrels (Doussot et al., 2002).

Furanic compounds, produced by depolymerisation of hemicelluloses and cellulose, are mainly formed during the toasting process and are related to sweet notes but also contribute with toasted almond aromas (Independent Stave Company, 1998). These compounds begin to emerge when the wood reaches 105 – 110 ºC, but start to degrade from 170 ºC, with maximums being found at around 140 – 150 ºC. Hemicelluloses are the most thermosensitive polymer of wood (Chatonnet & Boidron, 1989) as they degrade in the 130 – 194 ºC temperature range. When wood is heated, the pentoses, which are the main constituents of hemicelluloses, produce furfural, while 5-methylfurfural and 5-hydroxymethylfurfural are formed from the hexose units, which are the minor constituents of hemicelluloses and the main constituents of the most crystalline cellulose (Cutzach, Chatonnet, Henry, & Dubourdieu, 1999). 5-hydroxymethylfurfural values were much higher than those reported by Jordao, Ricardo-da-Silva & Laureano (2005) for fine grain *Quercus petraea* Liebl. and also by (Cadahía et al., 2003), both after a natural wood seasoning process. However, although higher, these values were closer to those reported by Cadahía, Fernández de Simón, Vallejo, Sanz, & Broto (2007) in *Q. petraea* wood. These compounds, after toasting, increased their concentration (Figures 2B, 2C and 2D), indicating the significance of toasting in the final content of the compounds derived from polysaccharides. According to the secondary wall structure, the LW wood zone has a larger central layer called S2 and therefore is richer in cellulose than the EW zone (Hamada et al., 2016, 2017). In this study, the staves classified as L-OTR presented an average LW area percentage slightly larger than the H-OTR ones, coinciding with previous work (Nevares et al. 2019), in which the woods with lower OTR rates also showed a greater LW area percentage value than those classified as H-OTR. Therefore, as the wood classified as L-OTR presented a slightly higher LW percentage, this could suggest that they were woods with a higher cellulose content, so logically more compounds originating from the degradation of polysaccharides were generated during the toasting process (Hamada et al., 2016, 2017), that is, furfural and derivates.

A decrease in the concentration of these compounds was to be expected in the wood samples taken from the inner surface of the staves (samples C) according to work mentioned previously, as the maximum temperature reached was 180 ºC. However, the mean values found in samples C (5 mm) were the highest in comparison with those obtained at the other points. In turn, the increment observed in the outer thickness of the toasted staves (~ 18.5 mm), in comparison with the untoasted samples was around 50 % in the case of 5-hydroxymethylfurfural for both types of staves and 90 % for furfural and 5-methylfurfural in L-OTR staves and 85 % in the H-OTR ones. The steady increase in the concentration of these compounds was a result of greater generation from cellulose and hemicelluloses in the face of thermal degradation of the furan compounds mentioned at temperatures close to 170 ºC, a hypothesis already confirmed by other authors (Chatonnet & Boidron, 1989) stating that these compounds were still being generated even at temperatures close to 220 ºC (Chatonnet, Cutzach, Pons, & Dubourdieu, 1999).

Lignin consists of two central structural parts: guaiacyl and syringyl. These structural units can begin to be released at temperatures close to 165 ºC (Hill, 2007), the resulted compounds being related to sweet and spicy aromas. According to Hill's work an increase in most of the compounds derived from lignin was observed in this study as a result of toasting. The effect of more intense toasting decomposes the lignin, first into phenolic aldehydes, mainly vanillin, syringaldehyde and other related compounds, and secondly, when temperatures reach values close to 180 – 190 ºC, volatile phenols such as guaiacol, ethyl phenol and cresols are generated (Independent Stave Company, 1998).

This was confirmed in this study as the compounds which increased greatly with toasting were syringaldehyde (Figure 2E) and vanillin (Figure 2F), direct products of the oxidative degradation of the two central structural parts (Doussot et al., 2002). Both compounds, related to vanillin aroma, start to be produced at 150 – 160 ºC, maximum at 195 – 215 ºC and degrade completely on toasting at temperatures above 250 ºC (Independent Stave Company, 1998). The highest temperature to which the staves were subjected in this study was 180 ºC, so an increase in their concentrations was to be expected. Vanillin and syringaldehyde were the most abundant compounds after 5-hydroxymethylfurfural and furfural ones in samples A, with higher concentrations in the H-OTR woods (approximately 6 µg/g for both compounds). In the case of L-OTR staves the toasting effect was more evident for both compounds (Figures 2E and 2F), with samples C presenting the highest content. Contrary to this, H-OTR staves showed the highest content in the outer thickness of the toasted staves (samples B). Hamada et al. (2016) recorded that the EW presented more lignin and hemicelluloses than the LW. As described in Table 1, H-OTR woods have a larger average EW area than L-OTR ones, which according to Hamada et al. (2016) indicates that H-OTR has more lignin and hemicelluloses. Therefore H-OTR woods can be expected to have higher concentrations of lignin and hemicelluloses derivate compounds, since the toasting process for H-OTR and L-OTR staves was the same. It is important to consider the study carried out by Collins et al. (2015), which demonstrated that the volatile composition of oak changed during the toasting process. Therefore the higher concentration of these compounds in H-OTR staves is accompanied by a higher degradation during toasting, although globally these woods are richer in these compounds.

In this study the wood toasting temperature was 180 ºC and a significant increase in the eugenol content was recorded, regardless of their OTR group. It is interesting to note that, as would be expected according to the generation and degradation temperatures previously indicated, the highest content of eugenol and isoeugenol was not in the most toasted zone C (Figures 2G and 2H). However, these results would corroborate those described by other authors who found that eugenol does not follow any specific pattern of accumulation or degradation during toasting, regardless of grain size (Collins et al., 2015). Its content also differs depending on the species and origin of the wood, but in general no great differences have been observed (Cadahía et al., 2003; Duval et al., 2013). As regards the compounds associated with smoke aromas (guaiacol and its derivates) and the rest of the volatile phenols, there is controversy about the generation and degradation temperatures: according to the Independent Stave Company (1998), wood needs to reach temperatures close to 190 – 200 ºC for those compounds to start to be formed, with the maximum being between 225 and 235 ºC. The studies by Chatonnet (1999) demonstrated that the concentrations of these compounds decreased to the levels found in wood prior to toasting at temperatures above 200 – 220 ºC. In general, no significant increases in concentration were observed in this group of compounds as a result of the toasting process (Figures 2I and 2J). However, when the degree of concentration inside the wood was compared according to the temperatures reached (samples A, B and C) for each group of staves (L-OTR and H-OTR), almost all the compounds showed significant differences.

Some compounds which are important for their sensorial contribution to coconut and wood aromas (Cadahía et al., 2003; Independent Stave Company, 1998) are the $\beta$-methyl-$\gamma$-octalactone isomers derived from lipids. The Independent Stave Company (1998) states that these compounds start to be generated from 95 ºC, their maximum being found at about 100 – 110 ºC, but they begin to degrade at over 150 – 160 °C. Nevertheless, increases in their concentration have been reported at over 200 – 220 ºC (Chatonnet, 1999). Watanabe & Sato (1971) established that the increase in the concentration of $\beta$-methyl-$\gamma$-octalactone isomers in toasted samples could be explained by oxidation of fatty acids or thermal degradation of the glycoside precursors of the lactones as recorded by Chatonnet (1995). Some years later (Wilkinson, Prida, & Hayasaka, 2013) established that degradation of the lactone precursors (which involves the generation of the free volatile of that compound) occurred after toasting at 200 ºC for 30 minutes. These papers indicated great heterogeneity in the lactone generation pattern as a result of the toasting process. So, in agreement with the bibliography, toasting treatments have a very different effect on the levels of these lactones: no effect (Fernández de Simón et al., 2009; Jordao et al., 2005), decrease with temperature (Collins et al., 2015; Fernández de Simón et al., 2009) or increase and then decrease (Farrell et al., 2015). Despite the fact that these compounds are present in all types of oak and more abundantly in untoasted woods, both isomers have been quantified in larger quantities in American White Oak (*Quercus alba* L.) in comparison with European Oaks (*Quercus petraea* L. and *Quercus robur* L.).

The high standard deviation showed by the two $\beta$-methyl-$\gamma$-octalactone isomers in the different samples (A, B and C) for both L-OTR and H-OTR staves reflects great variability in the content of these compounds in the staves, as previously observed by Duval et al. (2013) among trees. Therefore the variability inherent to wood was confirmed and also reflected in the differences found in the concentration of these compounds in the toasted staves studied (Collins et al., 2015; Duval et al., 2013; Farrell et al., 2015; Jordao et al., 2005). These high standard deviation values for both isomers meant that no significant differences were observed between the different samples in each OTR group since the mean values recorded indicated a higher concentration of the *cis* isomer and a lower one of *trans*-$\beta$-methyl-$\gamma$-octalactone in the L-OTR woods (Figures 2L and 2M).

The $\beta$-methyl-$\gamma$-octalactone isomers concentration profile differed depending on the OTR of the staves according to the toasting process carried out for this study. This profile presented a bell form in the L-OTR staves when the concentration gradient inside the wood was depicted according to the toasting temperature (Figures 2L and 2M). This behaviour coincided with the data provided by the Independent Stave Company (1998) and Collins et al. (2015), since the maximum concentration was recorded in the outer thickness of the toasted staves (samples B), where the maximum temperature reached was 135 ºC. As expected, the *cis*-*β*-methyl-*γ*-octalactone concentration was higher than that of the *trans* isomer in all the staves. However, the *cis/trans* relation was very different in both L-OTR and H-OTR staves with values between 1 and 10, which means that, although *cis*-*β*-methyl-*γ*-octalactone presented a higher content than *trans*-*β*-methyl-*γ*-octalactone, the ratio between them was very different depending on the staves. The heterogeneity related to the ratio value of lactones coincided with the values reported in the bibliography (Cadahía et al., 2007). As for the *γ*-butyrolactone, despite the concentrations on the inner surface of the stave and the untoasted samples being practically the same regardless of the OTR (Table 2), increases of this compound during toasting of the barrels have been mentioned in the literature (Cadahía et al., 2003; Fernández de Simón et al., 2009), stating that they were greater in American oak when compared with those detected in French or Spanish oak, thus demonstrating dependence on wood origin (Duval et al., 2013).

In general, the volatile content shown by the fresh woods (Figure 2) (samples A) was relatively low in comparison with other untoasted French oak (Cadahía et al., 2007; Doussot et al., 2002). The ANOVA statistical test carried out for the compounds analysed in samples A (Table 2) revealed that the content of some differed depending on the wood's OTR. Differences were observed in the phenolic volatile compound content and, to a lesser extent, the lactone content in the different OTR woods. The L-OTR woods had a significantly higher 4-ethylphenol and 4-methylguaiacol content, while H-OTR ones presented higher concentrations of guaiacol, *trans*-*β*-methyl-*γ*-octalactone and 4-ethylguaiacol. The composition of samples from the inner thickness of the toasted staves (samples C) meant that the concentration of most of the compounds studied increased in comparison with the fresh woods (samples A) (Table 2 and Figure 2). This increase was significant in 9 of the 16 compounds studied, though not the same ones in the high and low oxygenation staves, so the volatile phenols showed more significant differences in the L-OTR staves, while the phenolic aldehydes in H-OTR showed no significant differences (Table 2). On comparing the most toasted zones of the staves with different OTRs, which came into contact with the wine (samples C), their composition was found to be significantly different for the three furan compounds (furfural, 5-methylfurfural and 5-hydroxymethylfurfural), the phenylketone acetovanillone, the phenolic aldehydes (syringaldehyde and vanillin), two of the volatile phenols (4-ethylguaiacol and 4-ethylphenol) and the *trans*-*β*-methyl-*γ*-octalactone isomer. That meant an increased concentration and a change of significance with regard to the differentiation previously found in comparison with the A samples with a different OTR since, in the case of the C samples (inner thickness of the toasted staves), more compounds were reported for distinguishing the woods with a different oxygen transmission rate than samples A (Table 2). These results also showed that, even though from the same batch, origin and with similar anatomical characteristics (Table 1), but with a different OTR, the profile of the concentration gradient after toasting the staves was significantly different in some of the studied compounds. Moreover, it was confirmed that the formation of these volatile compounds and the exact depth to which their concentration was maximum, depended on temperature (Collins et al., 2015; Independent Stave Company, 1998).

### *4.3. Oak wood and wine differentiation*

Discriminant analysis allowed the wines and oak wood samples from high and low oxygen transmission rate groups to be differentiated based on their volatile composition, placing the wines aged in barrels with different OTRs on the Function 1 axis from positive to negative values. Besides, the Function 2 was able to differentiate the samples from woods with different OTR levels, separating high OTR samples on the positive axis and low OTR samples on the negative one (Figure 3). The wines aged in barrels with a low OTR were placed to the positive side of Function 1, being defined by higher concentration levels of 4-ethylguaiacol, eugenol, 4-vinylguaiacol, guaiacol and 4-methylguaiacol (variables with a positive coefficient), while wines aged in barrels with a high OTR were placed on the negative axis and defined by a greater content of *cis*-$\beta$-methyl-$\gamma$-octalactone (Table 3). On the other hand, Function 2 allowed differentiation of wood samples with high and low oxygen transmission rates. H-OTR staves were located on the positive side of this axis, which explains around 36 % of the variance and this was defined by 4-ethylguaiacol and *trans*-$\beta$-methyl-$\gamma$-octalactone, whereas L-OTR staves were located on the negative side and were defined by greater 4-ethylphenol, 5-hydroxymethylfurfural, 4-methylguaiacol, furfural and 5-methylfurfural content (Table 3). So, according to the results obtained for wood (Table 2), it is possible to observe some similar composition among those volatiles that were present, at higher or lower concentrations, in the oak wood staves classified according to their OTR and in the wine aged in barrels with different oxygenation rates. Therefore wines aged in barrels made of wood classified according to OTR (Martínez-Martínez et al., 2019) were differentiated by the oxygen received during ageing (Prat-García et al., 2020), but also by their volatile composition, which is related to the structural characteristics of the wood, as indicated in the previous sections.

## **5. Conclusions**

Fresh staves of *Quercus petraea* Liebl. wood from the same batch showed no statistically significant differences in their anatomical characteristics. However, they did have statistically significant differences in their oxygen transmission rate (low and high OTR staves) and presented a different volatile composition after toasting. This process affected the L-OTR wood composition more, presenting a higher furfural, 5-methylfurfural and 5-hydroxymethylfurfural, vanillin, acetovanillone and syringaldehyde content than the H-OTR ones. In contrast a higher concentration of *trans*-$\beta$-methyl-$\gamma$-octalactone and 4-ethylguaiacol compounds was found in H-OTR woods. These results corroborate that woods with a different OTR, classified by their main anatomical properties, have a significantly different volatile composition after toasting. When a red wine is aged in barrels made of *Quercus petraea* Liebl. wood with different OTRs, wines aged in L-OTR barrels are characterised by 4-ethylguaiacol, eugenol, 4-vinilguaiacol, guaiacol and 4-methylguaiacol while H-OTR wines are described by *trans* and *cis*-$\beta$-methyl-$\gamma$-octalactone.

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## Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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**Table 1.** Wood anatomical characteristics (mean and standard deviation)

| | ρ | OTR | EW | LW | G |
|---|---|---|---|---|---|
| L-OTR | 0.690 ± 0.097 a | 0.004 ± 0.003 a | 46.48 ± 11.62 a | 47.16 ± 9.64 a | 2.14 ± 0.49 a |
| H-OTR | 0.672 ± 0.033 a | 0.075 ± 0.012 b | 48.46 ± 6.93 a | 46.15 ± 6.84 a | 1.54 ± 0.23 a |

*ρ: density (g/cm$^3$); OTR: oxygen transmission rates (mg/L·day); EW: earlywood (% of area); LW: latewood (% of area); G: grain (mm)*

**Table 2.** ANOVA results of volatile compounds in seasoned (samples A) and toasted 10 mm (samples C) French oak staves classified according to their oxygen transmission rate (OTR) as low and high

| | Among Seasoned and Toasted 10 mm | | | | Among OTR groups | | | |
|---|---|---|---|---|---|---|---|---|
| | L-OTR | | H-OTR | | Seasoned | | Toasted 10 mm | |
| | **F** | ***p-value*** | **F** | ***p-value*** | **F** | ***p-value*** | **F** | ***p-value*** |
| ***Furanic compounds*** | | | | | | | | |
| 5-Hydroxymethylfurfural | 31.27 | ***0.0000*** | 25.37 | ***0.0000*** | 1.09 | *0.3023* | 10.11 | ***0.0029*** |
| Furfural | 35.90 | ***0.0000*** | 75.46 | ***0.0000*** | 0.23 | *0.6330* | 6.96 | ***0.0120*** |
| 5-Methylfurfural | 26.95 | ***0.0000*** | 39.81 | ***0.0000*** | 1.62 | *0.2107* | 4.62 | ***0.0381*** |
| ***Phenylketone*** | | | | | | | | |
| Acetovanillone | 0.51 | *0.4783* | 2.25 | *0.1415* | 0.01 | *0.9212* | 6.36 | ***0.0160*** |
| ***Phenolic aldehydes*** | | | | | | | | |
| Syringaldehyde | 19.81 | ***0.0001*** | 2.74 | *0.1060* | 0.15 | *0.6969* | 11.07 | ***0.0020*** |
| Vanillin | 40.01 | ***0.0000*** | 2.21 | *0.1454* | 0.52 | *0.4736* | 10.76 | ***0.0022*** |
| ***Volatile phenols*** | | | | | | | | |
| Eugenol | 5.01 | ***0.0312*** | 9.51 | ***0.0038*** | 0.69 | *0.4103* | 1.41 | *0.2420* |
| Isoeugenol | 0.88 | *0.3544* | 3.71 | ***0.0616*** | 0.06 | *0.8151* | 0.30 | *0.5871* |
| Guaiacol | 6.49 | ***0.0150*** | 0.51 | *0.4801* | 5.36 | ***0.0260*** | 0.23 | *0.6313* |
| 4-Ethylguaiacol | 1.04 | *0.3147* | 2.04 | *0.1617* | 5.75 | ***0.0215*** | 4.89 | ***0.0331*** |
| 4-Ethylphenol | 0.14 | *0.7101* | 3.17 | ***0.0831*** | 11.33 | ***0.0018*** | 8.8 | ***0.0051*** |
| 4-Methylguaiacol | 10.59 | ***0.0024*** | 70.57 | ***0.0000*** | 14.37 | ***0.0005*** | 0.13 | *0.7196* |
| 4-Vinylguaiacol | 0.00 | *0.9687* | 3.32 | ***0.0763*** | 2.68 | *0.1100* | 0.04 | *0.8487* |
| ***Lactones*** | | | | | | | | |
| *trans*-$\beta$-Methyl-$\gamma$-octalactone | 0.87 | *0.3578* | 0.10 | *0.7580* | 3.10 | ***0.0862*** | 4.47 | ***0.0412*** |
| *cis*-$\beta$-Methyl-$\gamma$-octalactone | 0.73 | *0.3968* | 0.26 | *0.6110* | 0.48 | *0.4913* | 0.05 | *0.8166* |
| $\gamma$-Butyrolactone | 26.75 | ***0.0000*** | 17.14 | ***0.0003*** | 0.15 | *0.6989* | 4.57 | *0.1442* |

*L-OTR: staves classified as low oxygen transmission rate; H-OTR: staves classified as high oxygen transmission rate.*

**Table 3.** Discriminant functions of discriminant analysis performed with the volatile compounds of the different width positions from the staves with low (L) and high (H) oxygen transmission rate (OTR) and from the wines aged in both barrel oxygenation levels (H-OTR and L-OTR) for three months.

| | ***Function 1 (44.60 %)*** | ***Function 2 (36.18 %)*** |
|---|---|---|
| 5-Hidroxymethylfurfural | -0.0072 | -0.2648 |
| Furfural | 0.0902 | -0.1643 |
| 5-Methylfurfural | 0.0600 | -0.1484 |
| Acetovanillone | 0.0418 | -0.0647 |
| Syringaldehyde | 0.1368 | -0.0806 |
| Vanillin | 0.0740 | -0.0584 |
| Eugenol | 0.1985 | 0.1123 |
| Isoeugenol | -0.0146 | 0.0350 |
| Guaiacol | 0.1778 | 0.1102 |
| 4-Ethylguaiacol | 0.2041 | 0.1940 |
| 4-Ethylphenol | 0.2235 | -0.3968 |
| 4-Methylguaiacol | 0.1625 | -0.1901 |
| 4-Vinylguaiacol | 0.1794 | 0.0355 |
| *trans*-*β*-Methyl-*γ*-octalactone | 0.0117 | 0.1612 |
| *cis*-*β*-Methyl-*γ*-octalactone | -0.1579 | -0.0696 |
| γ-Butyrolactone | -0.0270 | -0.1347 |

## Figures

**Figure 1.** Experimental design for wood sampling.

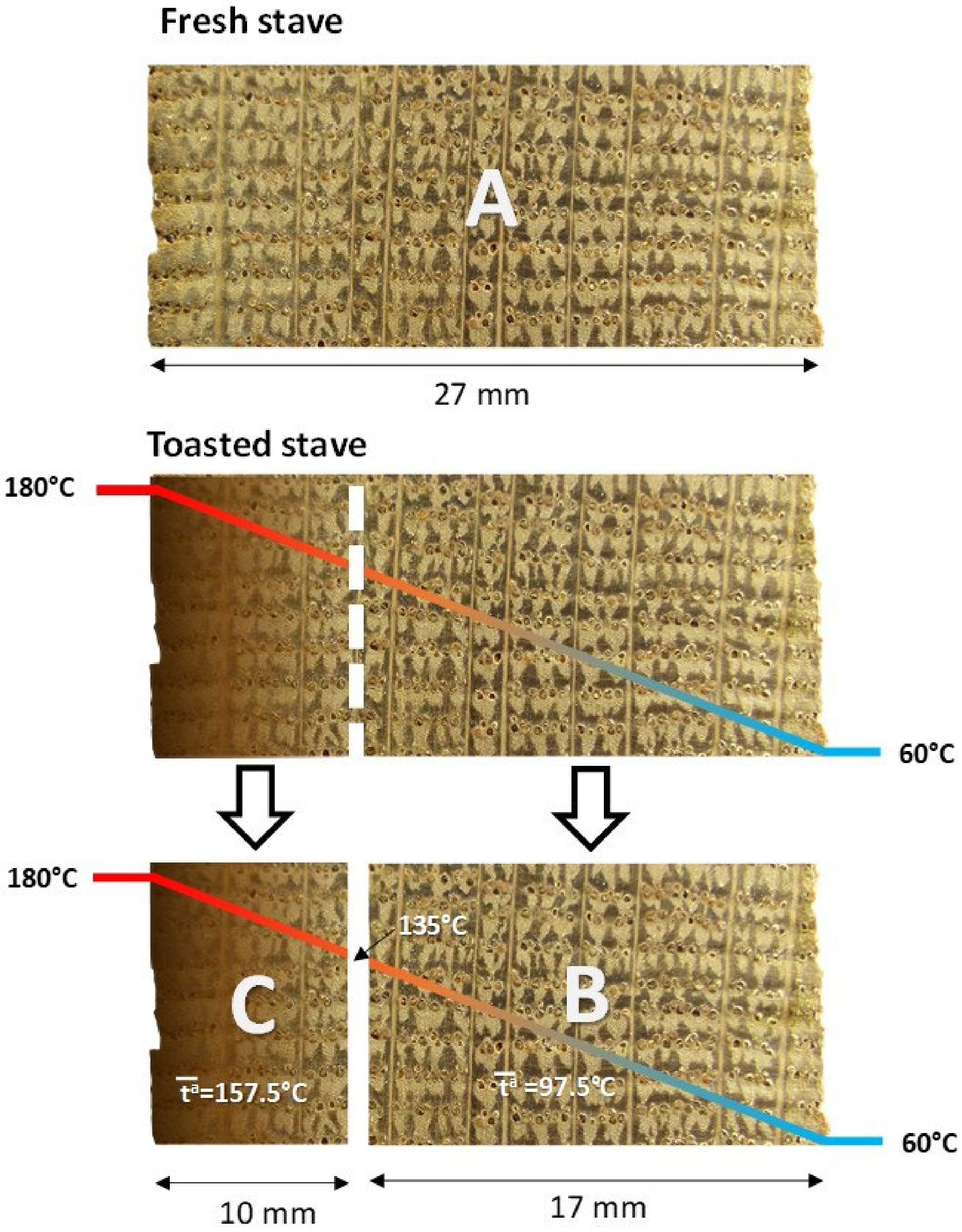
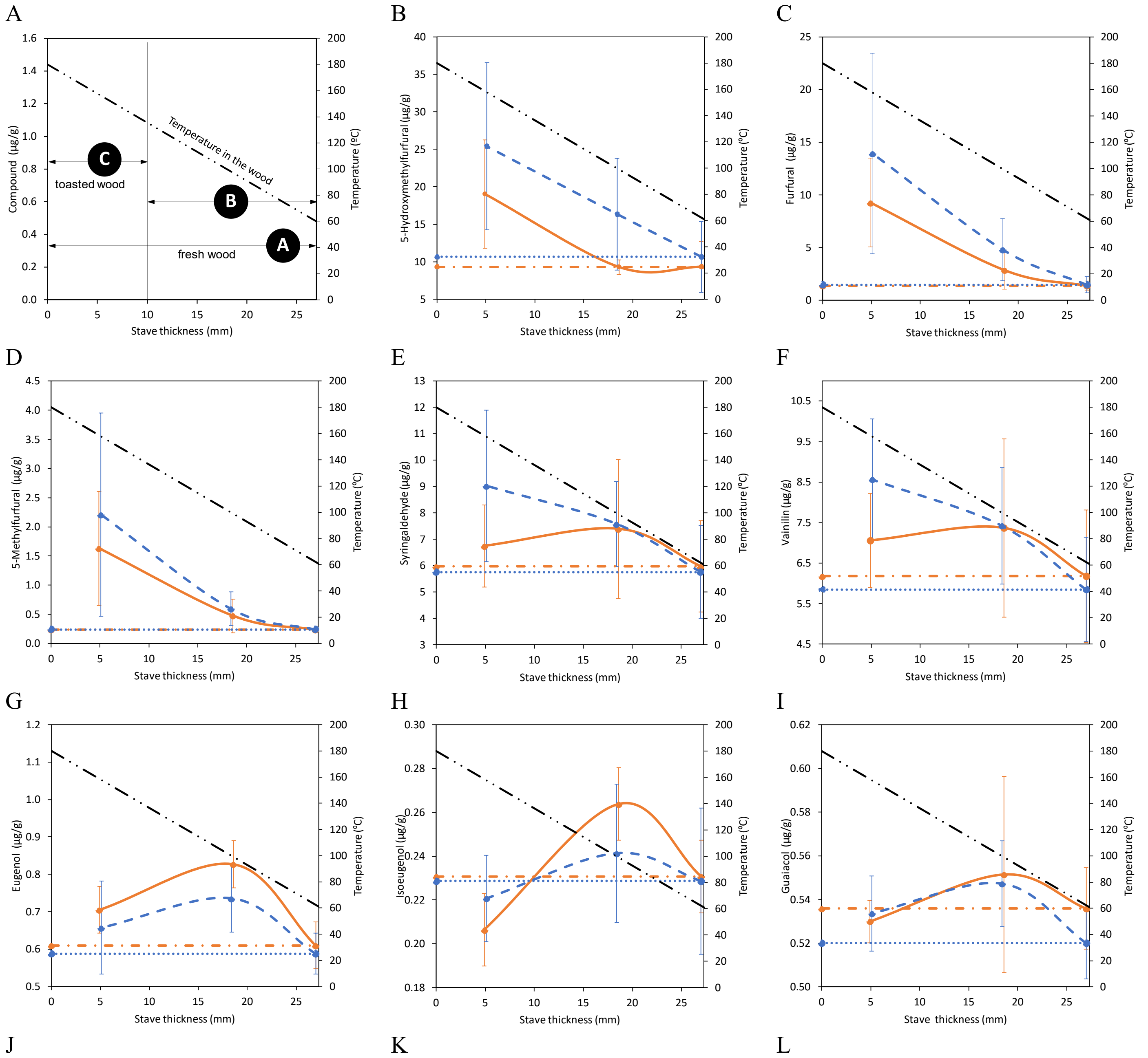


**Figure 2.** Concentration gradient of volatile compounds within the thickness of the staves A) Scheme concentrations; B) 5-Hydroxymethylfurfural; C) Furfural; D) 5-Methylfurfural; E) Syringaldehyde; F) Vanillin; G) Eugenol; H) Isoeugenol; I) Guaiacol; J) 4-Methylguaiacol; K) 4-Vinylguaiacol; L) trans-β-methyl-γ-octalactone; M) cis-β-methyl-γ-octalactone; N) γ-Butyrolactone.



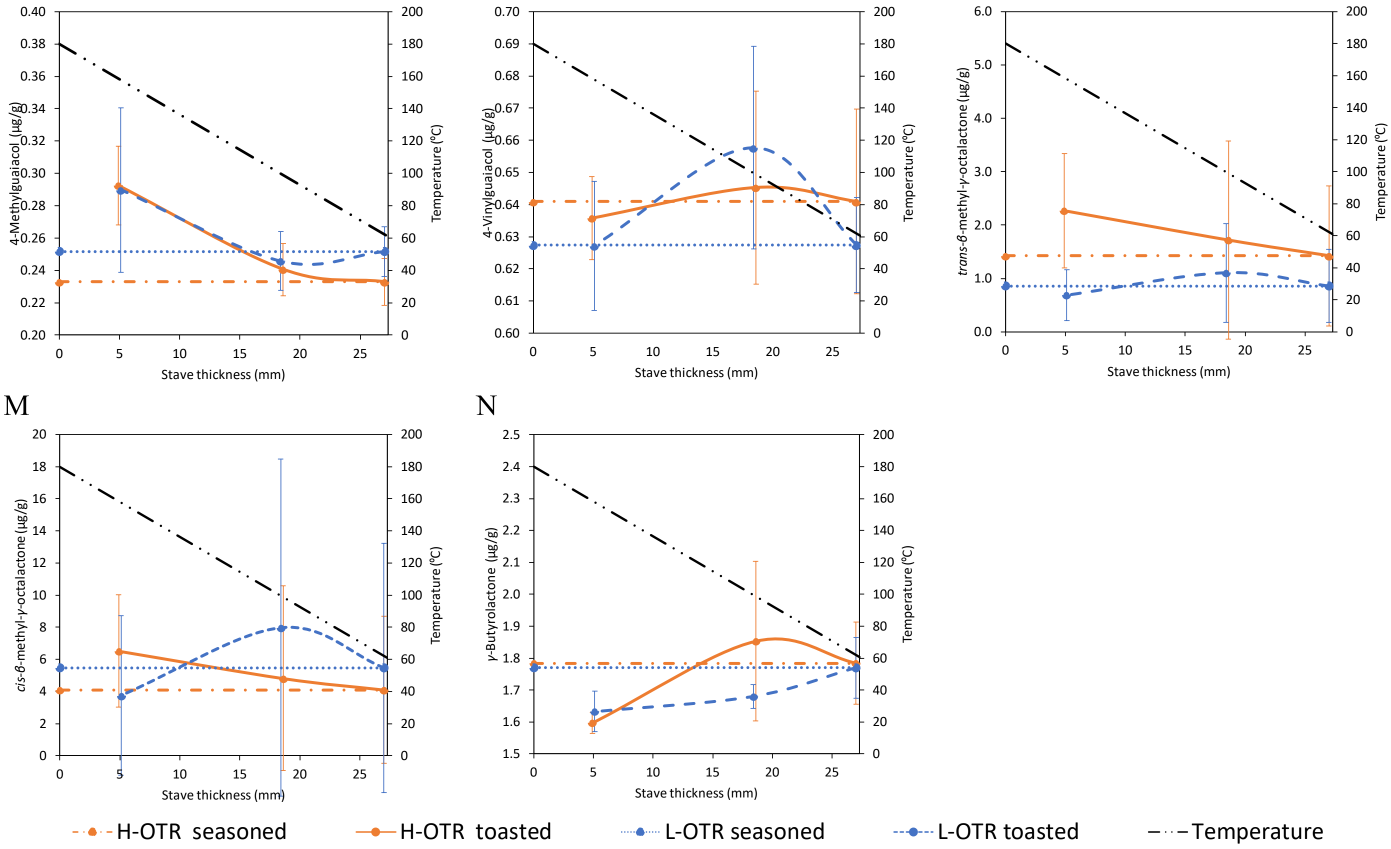

0.40
0.38
0.36
0.34
0.32
0.30
0.28
0.26
0.24
0.22
0.20
4-Methylguaiacol (µg/g)
200
180
160
140
120
100
80
60
40
20
0
Temperature (ºC)
0
5
10
15
20
25
Stave thickness (mm)
0.70
0.69
0.68
0.67
0.66
0.65
0.64
0.63
0.62
0.61
0.60
4-Vinylguaiacol (µg/g)
Temperature (ºC)
Stave thickness (mm)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
trans-β-methyl-γ-octalactone (µg/g)
Temperature (ºC)
Stave thickness (mm)
M
20
18
16
14
12
10
8
6
4
2
0
cis-β-methyl-γ-octalactone (µg/g)
Temperature (ºC)
Stave thickness (mm)
N
2.5
2.4
2.3
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
γ-Butyrolactone (µg/g)
Temperature (ºC)
Stave thickness (mm)
H-OTR seasoned
H-OTR toasted
L-OTR seasoned
L-OTR toasted
Temperature

**Figure 3.** Discriminant analysis performed with the volatile compounds of the different width positions from the staves with low oxygen transfer rate (*LA*: samples A, *LB*: samples B, *LC*: samples C) and high oxygen transmission rate (*HA*: samples A, *HB*: samples B, *HC*: samples C) and from the wines aged in low oxygen transfer rate barrels (L-OTR) and high oxygen transfer rate barrels (H-OTR)