

## Effect of Toasting Intensity and Wood Grain on Polyphenolic Compounds and Aroma Components in Oak (*Quercus petraea*) Heartwood

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**Abstract:** It is important for winemakers to understand the chemical constituents of oak heartwood used for barrels as different grain types and toasting regimes influence wine flavor. Changes induced by different heating regimes in low-molecular-weight (LMW) phenolics and volatile compounds of oak heartwood of different grain types were evaluated. The results showed that a higher temperature during the toasting process changed the chemical compositions of the heartwood and increased the amounts of several key soluble and volatile compounds as a result of various heat-induced reactions. Moreover, independent of toasting, the fine-grained oak samples had higher concentrations of volatile compounds, e.g., *cis/trans*-oak-lactone, *cis/trans*-isoeugenol, furanic aldehydes, syringol, and LMW phenolics, particularly sinapaldehyde, coniferaldehyde, vanillin, and syringaldehyde, while the medium- and coarse-grained oak samples showed higher levels of total phenol, tannins, ellagic acid and gallic acid. Principal component analysis (PCA) showed that the moderately and heavily toasted samples of medium- and fine-grained oak displayed higher positive correlations with the level of volatile compounds, while the untoasted and lightly toasted heartwood of coarse-grained oak were positively correlated with total tannin and ferulic acid contents.

**Key words:** oak heartwood; grain type; toasting level; phenolic compounds; volatile compounds

## 烘烤强度和木纹理对橡木 (*Quercus petraea*) 心材多酚和香气的影响

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**摘要:** 考察不同烘烤程度对不同纹理橡木心材试样中低分子质量酚酸和挥发性物质含量的影响, 结果表明烘烤过程将会改变橡木心材的物质组成, 并通过相应的化学反应增加挥发性物质的含量。同时, 在不考虑烘烤强度条件下, 供试的细纹理橡木心材试样含有较高的挥发性物质含量, 例如顺/反橡木内脂、顺/反异丁子香酚、呋喃醛、紫丁香醇, 以及芥子醛、松柏醛、香草醛和丁香醛等低分子质量酚类化合物, 而中纹理和粗纹理的样品则表现出较高的总酚、总单宁水平, 以及较高的鞣花酸和没食子酸含量。此外, 主成分分析显示, 中纹理和细纹理样品的中度和重度烘烤处理与橡木心材试样的挥发性物质含量有较大的正相关性, 而粗纹理的未烘烤和轻度烘烤样品则与试样中总单宁和阿魏酸含量呈正相关。

**关键词:** 橡木; 纹理; 烘烤度; 酚类物质; 挥发性物质

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Aging wine in oak barrels is a traditional vinification practice employed by winemakers for improving wine quality in particular<sup>[1]</sup>. During barrel aging wines undergo a series of complex processes, including extraction of many substances from the wood matrix<sup>[2]</sup>, in part, due to the slow pick-up of oxygen permeating through the slightly porous wood<sup>[3]</sup>. Also there is steady clarification while in the barrel<sup>[4]</sup>. These changes modify the color and tannin profile of the wine and thus, contribute to the development of the wine's sensory profile.

In general, American white oak (*Quercus alba*) and French red oak (*Q. robur* and *Q. petraea*) are the most commonly used species for wine barrels and have been used as for thousands of years<sup>[5]</sup>. The basic compositions of oak are not significantly different from one species to another. Cellulose (40%) and hemicellulose (25%), which comprise the structure of the heartwood, are the major constituents of oak, while lignin, a phenolic polymer, is present mainly in the cell walls, accounts for 20% of the dry weight<sup>[1,6]</sup>. However there are also important minor components, such as terpenes and lipids, which may comprise up to 0.5% on a weight basis. Some are volatile and when extracted into a wine, they contribute an oaky note to the sensory profile of the wine<sup>[7]</sup>. The low-molecular-weight (LMW) phenolics and oak tannins affect color and astringency of the wines. Thus the extraction of a wide variety of compounds from the oak during the barrel aging has been considered as to contribute positively to wine quality<sup>[8]</sup>.

Previous studies have shown that the concentrations of oak compounds in aged wine depended on the amount of the potential extractable constituents originally present in the barrels' wood and the aging conditions<sup>[1,6]</sup>. Furthermore, factors such as geographical origin<sup>[9-10]</sup>, silvicultural practices<sup>[5]</sup>, and barrel production process<sup>[11]</sup> all influence the composition of the wood and are directly responsible for the final sensory character of the wine.

Among these factors, wood heat treatment is a major factor determining the sensory and physicochemical modifications of a wine during barrel aging<sup>[12]</sup>. During the heat treatment, diverse hydrothermolysis and pyrolysis reactions occur, causing degradation of lignins, polysaccharides and lipids, and leading to the production of

numerous aromatic compounds such as furanic aldehydes<sup>[13]</sup>. Moreover, the thermal treatment of oak wood also reduces the level of tannins (ellagitannins) and increases the amount of ellagic acid<sup>[14-15]</sup>.

In the last few years, several studies have analyzed the contribution of oak wood to the chemical composition of barrel aged wine. However these investigations generally focused on aspects such as oak species, geographical sources, and processing methods<sup>[7,14,16-17]</sup>. The composition of aromatics and phenolics as a function of the wood grain has been rarely studied<sup>[18-19]</sup>.

Typically, coopers classify oak grain as one of three types: fine, medium and coarse, according to both the width of annual growth rings and the texture of wood. The grain properties would influence the wood chemical compositions and ultimately affect the contribution of barrel aging to wine quality<sup>[5]</sup>. In addition, other factors such as toasting at cooperage also affect the sensory and chemical characteristics of woods, especially their wood-related volatile composition<sup>[19]</sup>. Hence, it would be important to know more information about the chemical compositions of wood grains along with different toasting treatments. In this study, we have quantified the changes, induced by the toasting process, in the LMW phenolics and volatiles in oak (*Q. petraea*) heartwoods of different grain types. The cooperage industry needs to develop criteria for controlling raw materials<sup>[20]</sup> and also toasting regimes. Understanding these two parameters and how they interact will assist coopers and winemakers.

## 1 Materials and Methods

### 1.1 Wood samples and chemicals

The heartwood oak samples provided were the same as would be used as staves for making barrels by Yantai Demptos Oak Barrel Co. Ltd. (Yantai, Shandong, China). The selected oak wood was from 160-year old *Q. petraea* oak species grown in the Troancas forest located in the centre region of France. The raw wood was naturally seasoned for 24 months during which the moisture content dropped to 15%. Heartwood of three different grain tightness was selected: fine (1 to 2 mm), medium (2 to 4 mm) and coarse (4 to 5 mm). All staves were about 23 mm in thickness and toasted in our

laboratory oven according to the method of Ribereau-Gayon and coworkers with some appropriate modifications<sup>[21]</sup>: light (150 °C for 15 min), medium (175 °C for 15 min) and heavy (200 °C for 20 min). Heartwood samples were taken from the untoasted and toasted staves, and four staves of each grain type and toasting degree combination were used. The heartwood pieces were cut from the center and the extremities of these staves. From toasted stove samples, a layer of toasted heartwood was cut off to a depth of 4 mm. The heartwood pieces were ground and sieved, the sawdust ranging from 0.28 to 0.80 mm in size were taken.

These standards, included ellagic acid (CAS No.: 476-66-4), gallic acid (149-91-7), vanillic acid (121-34-6), syringic acid (530-57-4), ferulic acid (1135-24-6), coniferaldehyde (20649-42-7), syringaldehyde (134-96-3), sinapaldehyde (4206-58-0), *o*-vanillin (148-53-8), vanillin (121-33-5), 3,4-dimethylphenol (95-65-8), eugenol (97-53-0), phenol (108-95-2), *o*-guaiacol (90-05-1), 4-vinylguaiacol (7786-61-0), *cis/trans*-isoeugenol (5912-86-7/97-54-1), syringol (91-10-1),  $\gamma$ -caprolactone (695-06-7), *cis/trans*-oak-lactone (55013-32-6/39638-67-0), furfural (98-01-1), 5-methylfurfural (620-02-0), and 5-hydroxymethylfurfural (67-47-0) were supplied by Sigma. Deionised water (<18 M $\Omega$  resistance) was obtained from a Milli-Q Element water purification system (Millipore, Bedford, MA, USA). HPLC grade acetic acid and methanol were obtained from Honeywell (Burdick & Jackson, USA). All the other chemicals (analytical grade) were obtained from Beijing Chemical Co. Ltd. (Beijing, China), unless noted specially.

## 1.2 Instruments and equipments

1100 MSD HPLC-MS with a DAD UV-Vis detector coupled to a mass spectrometer (Trap-VL) and equipped with an ESI interface (Agilent, Palo Alto, CA, USA); C<sub>18</sub> steel column (250 mm  $\times$  4.0 mm i.d., 5  $\mu$ m) was obtained from Zorbax SB, Agilent (USA); 7890A/5975C GC-MS, Innovax fused-silica capillary column (60 m  $\times$  0.25 mm i.d., 0.25  $\mu$ m film thickness) was also obtained from Agilent (USA); UV-2450 UV-Visible spectrophotometer was purchase from Shimadzu Corporation (Kyoto, Japan).

## 1.3 Methods

### 1.3.1 Extraction of phenolic compounds

The phenolic compounds were extracted based on the method previously described by Sanz et al.<sup>[22]</sup>. A sample of 1 g of each was extracted with 100 mL of methanol/water (1:1) at room temperature and in darkness for 24 h. Then, the extracts were filtered in a Büchner funnel, and the methanol

in the extracts was removed in a rotary evaporator at below 40 °C. The aqueous solution obtained was extracted three times with 20 mL of diethyl ether and ethyl acetate. The two organic fractions were evaporated in a rotary evaporator at below 40 °C. The residues were re-dissolved in 5 mL of methanol solution for further analyses. All the extractions were carried out in triplicate.

### 1.3.2 Extraction of volatile compounds

Volatile compounds were isolated by making liquid-liquid extracts of the hydroalcoholic heartwood extracts, using the following protocol: 1 g of dry heartwood sawdust was soaked in 100 mL of 50% ethanol solution in a glass vial fitted with an elastomer stopper. The vial was heated for 30 min at 50 °C in a shaking table to accelerate the diffusion of the heartwood's volatile compounds. After the mixture was filtered, 40  $\mu$ L of mixed internal standards (*o*-vanillin (2.002 mg/L solution in water), 3,4-dimethylphenol (1.027 mg/L solution in water) and  $\gamma$ -caprolactone (2.043 mg/L solution in water)) was added in every sample. Then the solution was extracted with 15 mL of dichloromethane, distributed three times. The organic fraction was dried on anhydrous sodium sulfate and reduced to 1 mL under nitrogen flux before being submitted to GC-MS analysis. In all cases, the samples were carried out in triplicate.

### 1.3.3 Determination of LMW phenolic compounds, total phenol, and total tannin

The LMW phenolic compounds were assessed by using HPLC-MS. Chromatographic separation was carried out by using C<sub>18</sub> steel column, maintained at 30 °C. The HPLC profiles were monitored at 255, 280, 325, and 340 nm, and the UV-Vis spectra were recorded from 190 to 700 nm. The injection volume was 10  $\mu$ L. The mobile phase, with a flow rate of 1 mL/min, consisted of a mix of solvents: A (water/acetic acid, 99:1 (V/V)) and B (methanol/acetic acid, 99:1 (V/V)) with following elution gradient: 0–3 min, 0% B in A; 3–25 min, 40% B in A; 25–43 min, 60% B in A; 43–55 min, 60% B in A; 55–60 min, 80% B in A; 60–65 min, 80% B in A; and 65–69 min, 0% B in A. The MS conditions were: ESI, negative ion mode; 35 psi nebulizer pressure; 10 mL/min dry gas flow rate, 350 °C dry gas temperature, and scanned at *m/z* 100–1 000. For the quantitative analyses, calibration curves were obtained by using standards of each LMW phenolic compounds, and were quantified as the corresponding compound. The measurements were taken in triplicate.

Folin-Ciocalteu and Folin-Denis procedures were used

to measure the total phenol and tannin respectively<sup>[23]</sup>. The quantitative data were expressed as mg/g of dry heartwood, and all the measurements were performed in triplicate.

#### 1.3.4 Determination of volatile compounds

Analyses were performed using an Agilent 7890A/5975C GC-MS equipped with a HP-Innowax fused-silica capillary column. Helium carrier gas was circulated at 1 mL/min in the constant flow mode. The split/splitless injector was used in the splitless mode. The injected volume was 1  $\mu$ L and the injector temperature was 250  $^{\circ}$ C. The oven temperatures were programed as follows: the oven temperature was programmed from 50  $^{\circ}$ C; 7  $^{\circ}$ C/min ramp to 120  $^{\circ}$ C and holding for 5 min; 2  $^{\circ}$ C/min raised to 200  $^{\circ}$ C; 10  $^{\circ}$ C/min raised to 240  $^{\circ}$ C and maintained for 20 min. The ion energy for electron impact (EI) was always set at 70 eV. The source temperature was 200  $^{\circ}$ C. Identification of the volatile compounds was made by comparing the GC retention times and mass spectra over the mass range 30–500 mass units for the samples with pure standards analyzed under the same conditions. Quantitative data of the identified compounds were measured from the characteristic ion peak areas with regard to the internal standard of *o*-vanillin for vanillin; 3,4-dimethylphenol for eugenol, phenol, *o*-guaiacol, 4-vinylguaiacol, *cis/trans*-isoeugenol, syringol; and  $\gamma$ -caprolactone for *cis/trans*-oak-lactone, furfural, 5-methylfurfural and HMF. Triplicate analyses were carried out.

#### 1.4 Statistical analysis

The data were analyzed by carrying out univariate analysis with ANOVA, by applying Fisher's least significant difference test (LSD). In addition, principal component analysis was applied to identify those variables that separate the different samples. All of the statistical analyses were performed by using SPSS software, version 20.0 (SPSS Inc., Chicago, IL, USA) program.

## 2 Results and Analysis

### 2.1 Effect of toasting intensity and wood grain on total phenol and tannin in oak heartwood

Table 1 lists the total phenols and tannins in the three different grain classes from the species *Q. petraea*. For the untoasted heartwood samples, the extractable total phenols were the highest in the coarse grain, followed by the medium and then the fine-grained samples. The total phenolic extraction was also influenced by the toasting process. The total phenols extracted rose to a maximum in the

medium toasted oak before decreasing to a lower level in the heavily toasted oak, except for the fine-grained oak turned to the highest value of total phenol at light toasting level. In particular, the medium toasted coarse-grained samples showed a 142% increase in the total extractable phenolic content over the untoasted material, while a 116% increase was observed in the medium-grained counterparts, and a 40% increase was observed in the fine-grained oak samples after light toasting. So while greater heat treatment to the medium toast level generated higher quantities of phenolics, intense toasting has been shown to carbonize lignins and hemicelluloses, and degrade other compounds, such as phenolic aldehydes, thereby decreasing the total phenolics<sup>[11]</sup>. This explains the decrease in total extractable phenolics at higher toasting intensity across all grain types.

**Table 1 Total phenol and tannin contents of oak heartwood after different toasting regimes**

Grain	Total phenol/(mg of gallic acid/g of heartwood)				Total tannin/(mg of tannic acid/g of heartwood)			
	Untoasted	Light	Medium	Heavy	Untoasted	Light	Medium	Heavy
Fine	49.7 <sub>a</sub> ±1.4	69.7 <sub>b</sub> ±2.5	61.9 <sub>b</sub> ±0.2	59.8 <sub>b</sub> ±0.2	1.86 <sub>a</sub> ±0.08	1.53 <sub>b</sub> ±0.05	0.81 <sub>c</sub> ±0.08	0.26 <sub>d</sub> ±0.08
Medium	50.5 <sub>a</sub> ±1.1	80.5 <sub>b</sub> ±0.9	110.1 <sub>b</sub> ±8.5	69.0 <sub>b</sub> ±8.5	2.04 <sub>a</sub> ±0.01	1.37 <sub>b</sub> ±0.06	1.00 <sub>b</sub> ±0.06	0.47 <sub>c</sub> ±0.05
Coarse	56.2 <sub>a</sub> ±2.2	85.6 <sub>b</sub> ±2.1	136.2 <sub>b</sub> ±3.3	96.9 <sub>b</sub> ±3.3	2.38 <sub>a</sub> ±0.03	1.69 <sub>b</sub> ±0.03	1.16 <sub>b</sub> ±0.04	0.53 <sub>c</sub> ±0.08

Notes: Average and standard deviation ( $\bar{x}\pm s$ ) were calculated for three samples. Values with different English letters (a–d) and Greek letters ( $\alpha$ – $\gamma$ ) within the same row and column indicate the significant differences according to the LSD ( $P<0.05$ ), respectively.

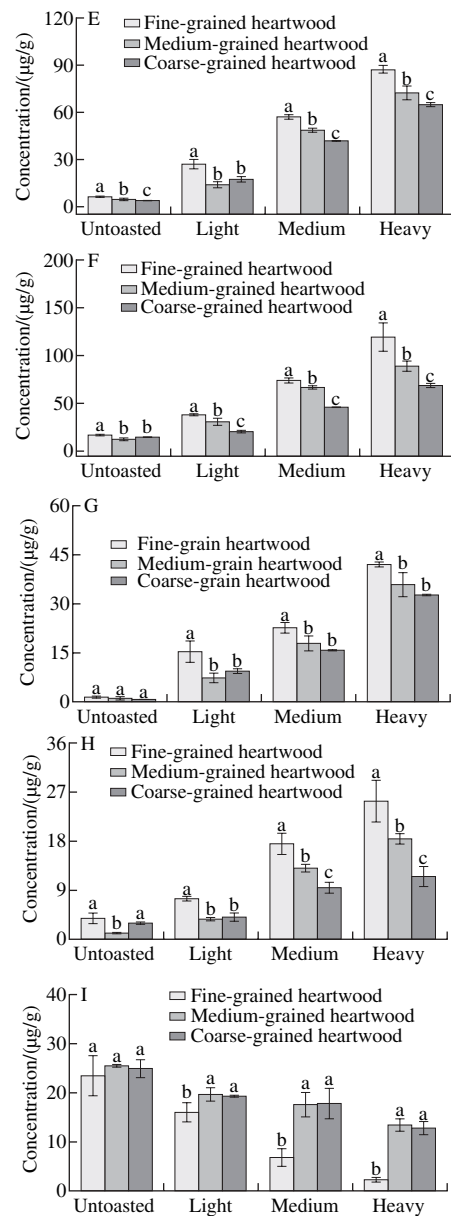
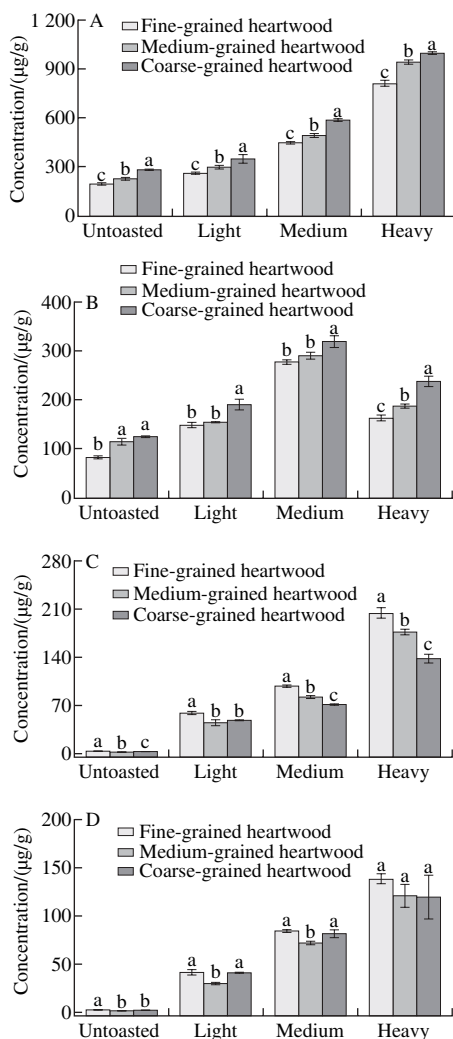
In contrast, the untoasted heartwood was rich in total tannin, which was in accord with Cadahia<sup>[15]</sup> and Glabasnia<sup>[24]</sup> et al.'s results which showed a predominance of hydrolyzable tannins (ellagitannins). Similar to the total phenols, total extractable tannins were the highest in coarse grain heartwood, and this trend was observed for all the heat treatments. However, in contrast to total phenols, total extractable tannins were decreased with increasing the heating intensity, independent of grain type. It has been previously reported that tannin accumulation was observed microscopically in parenchyma cell vacuoles, and the production might be related to the depletion of starch reserves. When the tannin accumulation reached a certain level, the vacuole was disrupted, thus mixing the tannins with the cytosolic constituents. Cell disintegration allowed the tannins to diffuse throughout the woody tissues<sup>[25]</sup>. Because the deposition of tannins occurred predominantly in large diameter spring growth vessels, the coarse-grained tissue of oak heartwood accumulated more tannin during the entire growth stage, explaining why the coarse-grained oak samples have the highest level of total tannin. Moreover, we found the tannins of coarse-grained sample were extremely sensitive



to heat treatment, and their concentration was decreased by 29% after light toasting, by more than 51% and 78% when the heartwood was subject to medium and heavy toasting, respectively. A similar trend was also observed in the medium-grained samples. In the fine-grained samples, the content was decreased by 18% to 86% with the increase in toasting intensity from light to heavy.

## 2.2 Effect of toasting intensity and heartwood grain on LMW phenolics in oak heartwood

HPLC analysis of the diethyl ether and ethyl acetate extracts revealed the presence of the following LMW phenolics in the oak heartwood samples: ellagic acid, gallic acid, vanillic acid, syringic acid, and ferulic acid. Of these sinapaldehyde, coniferaldehyde, syringaldehyde, and vanillin were identified before and after the toasting as shown in Fig. 1, and all the compounds were higher in concentration in the toasted samples compared with those of the untoasted ones.



A. Ellagic acid; B. Gallic acid; C. Sinapaldehyde; D. Coniferaldehyde; E. Vanillin; F. Syringaldehyde; G. Vanillic acid; H. Syringic acid; I. Ferulic acid. Bars represent the standard deviation. Values with different English letters (a-c) on the same toasting level are significantly different according to the LSD ( $P < 0.05$ ), Fig. 2 the same.

**Fig. 1 Evolution of concentrations of LMW phenolic compounds in toasted oak heartwoods with different grains**

As shown in Fig. 1, ellagic acid and gallic acid were the most abundant phenolic acids in these oak samples, even in the untoasted heartwood. Oak is known to be rich in ellagitannins; so it was not surprising that ellagic acid was found to be present at high concentrations ( $>190 \mu\text{g/g}$ ) among all sample extracts. It was found to be highest in the heavily toasted oak samples (coarse-grained  $996 \mu\text{g/g}$ , medium-grained  $948 \mu\text{g/g}$ , and fine-grained  $812 \mu\text{g/g}$ ), followed by medium-toasted oak

(584  $\mu\text{g/g}$  for the coarse grain, 492  $\mu\text{g/g}$  for the medium-grained, and 447  $\mu\text{g/g}$  for the fine-grained) ( $P<0.05$ ), with the lowest, 192  $\mu\text{g/g}$ , in the untoasted fine-grained sample (Fig. 1A). In all the samples, the ellagic acid content increased with toasting, probably due to the increasing thermolytic degradation of ellagitannin<sup>[26]</sup>. Moreover, the accumulation of ellagic acid can be also attributed to its high melting point, which prevents its carbonization even at toasting temperatures<sup>[15]</sup>.

Gallic acid is also important in oak heartwood, because during aging it contributes to astringency and bitterness, and acts as a copigment, enhancing the color of aging wine as well<sup>[27]</sup>. In our results, the levels of gallic acid were much higher in the coarse-grained samples than in the other grain types (Fig. 1B). An average value of 125  $\mu\text{g/g}$  gallic acid was found in the untoasted coarse-grained heartwoods, whereas 116 and 83  $\mu\text{g/g}$  gallic acid were found in the medium- and fine-grained oak samples, respectively. In addition, similar to ellagic acid, light and medium heat treatment caused drastic increasing changes in gallic acid content. As can be seen from Fig. 1B, the gallic acid content was higher in the medium-toasted heartwood (278  $\mu\text{g/g}$  for the fine-grained, 291  $\mu\text{g/g}$  for the medium-grained, and 320  $\mu\text{g/g}$  for the coarse-grained) being 2.3, 1.5 and 1.6 times as much as the original concentration in untoasted heartwood, respectively. This increase of gallic acid concentration agrees well with previous works<sup>[6,22]</sup>, and can be explained by thermolysis of galloyl esters associated with the parietal composites of the cells during the toasting process. However, a decrease in gallic acid content was observed after heavy toasting (decreased by 25% to 41% compared to medium-toasted oak samples). This could be explained by the extreme sensitivity of gallic acid to heat treatment<sup>[22]</sup>; therefore, the gallic acid content was gradually decreased with increasing toasting.

Apart from ellagic acid and gallic acid, phenolic aldehydes were the most abundant LMW phenolics in all of the heartwood extracts, with the fine-grained oak heartwoods having the phenolic aldehyde content higher than the other grain types. As shown in Fig. 1C, 1D, 1E and 1F, the untoasted heartwood had low levels of phenolic aldehydes (sinapaldehyde, coniferaldehyde, syringaldehyde and vanillin). According to previous studies, the degradation of lignin by heat affords mono- and di-methoxy hydroxyphenyl aldehydes<sup>[28]</sup>. Thus, the increase in contents of sinapaldehyde, coniferaldehyde, syringaldehyde, and vanillin in the toasted heartwood samples was to be expected. The data

showed that the concentration of sinapaldehyde increased significantly ( $P<0.05$ ) in the fine-grained samples during the toasting process, and the largest increase was observed after heavy toasting, being some 66 times that of the original concentration and 16% and 50% higher than those in the heavily toasted medium- and coarse-grained oak heartwoods, respectively. This higher value of sinapaldehyde in fine-grained toasted samples can be explained by a higher amount of di-methoxy hydroxyphenyl units in the fine-grained lignin structure, which could be thermo-depolymerized more easily than the mono-methoxy hydroxyphenyl units<sup>[29]</sup>. Compared to sinapaldehyde, the concentration of coniferaldehyde was lower, but both coniferaldehyde and sinapaldehyde displayed a similar trend in the samples of different grain types and toasting levels. It is well known that the angiosperm lignin is a three-dimensional heteropolymer formed by sinapyl and coniferyl alcohol copolymerization<sup>[30]</sup>. Thus, the anatomic tissue conformation of oak heartwood would be different for different grains. It was suggested that the fine-grained oak sample contained more lignin in its cell walls. During the heartwood toasting, the lignin underwent depolymerization reactions, while sinapyl and coniferyl alcohol were depolymerized and oxidised to the corresponding aldehydes. Therefore, the higher concentrations in fine-grained toasted oak heartwoods can be explained by the difference in the phenolic aldehyde content in different grain types and toasting levels. As the toasting intensity increased, sinapaldehyde and coniferaldehyde were produced by the cleavage of  $\alpha, \beta$  double bonds ( $\text{C}=\text{C}$ ) of cinnamic aldehydes, while thermo-oxidation and thermo-decarboxylation reactions produced mainly benzoic aldehydes such as syringaldehyde and vanillin<sup>[31]</sup>. In particular, the concentration of syringaldehyde increased from 14  $\mu\text{g/g}$  (untoasted level) to 92  $\mu\text{g/g}$  (heavy toasting level), accompanied by vanillin rising from 5  $\mu\text{g/g}$  to 75  $\mu\text{g/g}$ . In spite of this increase, the detected levels of these compounds were slightly lower than their sinapaldehyde and coniferaldehyde precursors, in toasted heartwood.

Vanillin has a relatively low flavor threshold (60  $\mu\text{g/L}$ ) and is associated with vanilla and coffee as well as dark chocolate and smoky odors<sup>[32]</sup>. During the toasting process, the content of vanillin was increased steadily with toasting intensity, with increases of 12, 15 and 18 folds in the fine to coarse grain samples, respectively. In addition the vanillin level in the heavily toasted medium- and coarse-grained oak samples were 17% and 26% lower than that in the fine-grained ones at the same toasting level. This trend was also observed for syringaldehyde, which has smoky and spicy aroma descriptors.

Previous studies have reported that the syringaldehyde/vanillin ratio is a very important index to evaluate oak heartwood and should be maintained between 1.4 and 2.5<sup>[33]</sup>. In this study, the fine- and medium-grained oak samples at light and medium toasting levels had syringaldehyde/vanillin ratios within these suggested limits, indicating that the products of lignin decomposition were balanced.

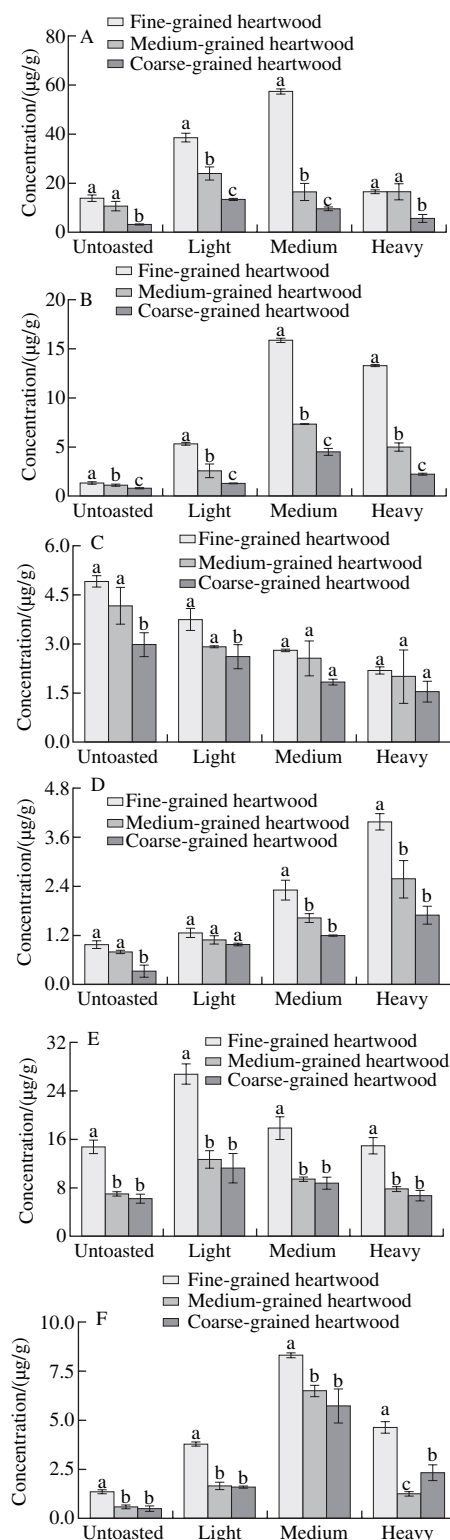
Other LMW phenolics in the oak samples extracts, including the derivatives of phenolic aldehyde, were also detected in the HPLC analysis. The vanillic acid and syringic acid (Fig. 1G and Fig. 1H) were produced by the oxidation of vanillin and syringaldehyde, and increased in level with toasting intensity. However, the content of ferulic acid was decreased due to its decarboxylation and degradation at a high temperature during toasting<sup>[6]</sup>, with the lowest levels in the fine-grained samples (Fig. 1I).

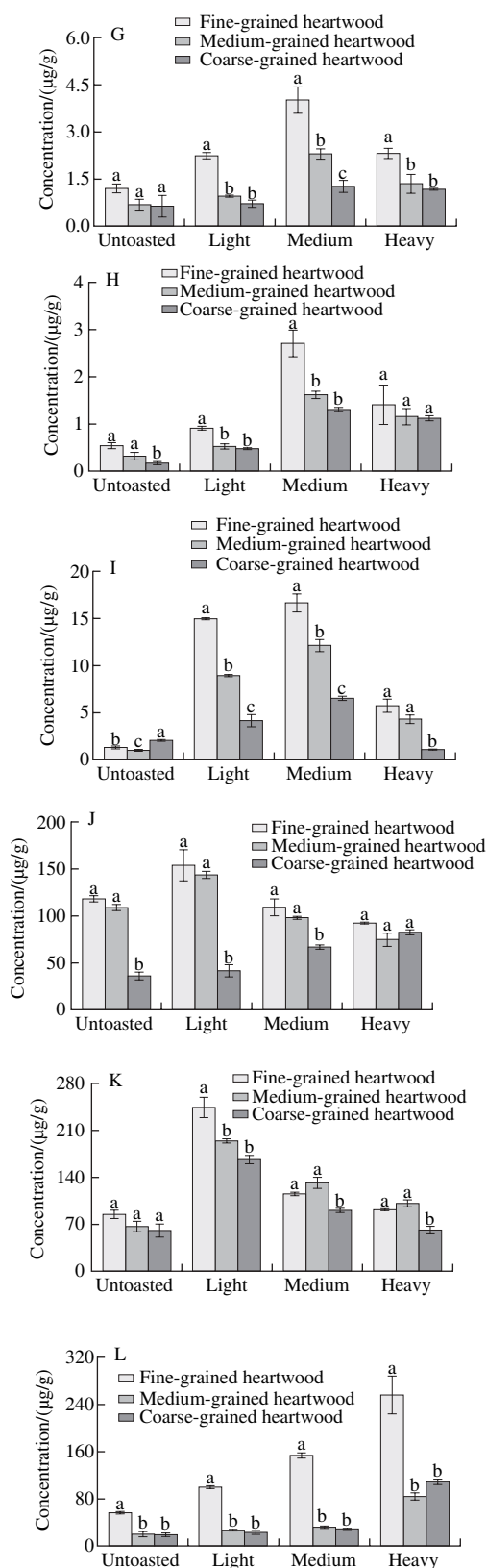
### 2.3 Effect of toasting intensity and heartwood grain on volatile compounds in oak heartwood

During heat treatment, the degradation of polysaccharides and polyphenols in oak gives rise to new volatile compounds that increase the amount of odoriferous substances<sup>[13]</sup>.

In this study (Fig. 2), two oak lactone isomers (*cis*- and *trans*- $\beta$ -methyl- $\gamma$ -octalactone) were identified in the oak and their amounts were significantly increased with medium heat treatment. For example, their average contents in the fine-grained oak samples ranged from 15  $\mu\text{g/g}$  in the untoasted heartwood to 73  $\mu\text{g/g}$  in the medium-toasted heartwood, but then decreased with heavy toasting (30  $\mu\text{g/g}$ ). Many studies have reported that toasting increases the levels of the oak lactones by releasing their precursor, the 6'-*O*-gallate derivative of  $\beta$ -D-glucopyranosyloxy-3-methyloctanoic acid<sup>[34-35]</sup>. However, we extracted more oak lactones in the light- and medium-toasted samples than in the heavy-toasted, possibly through the thermo-degradation of these heat-sensitive compounds or their loss by volatilization when the oak heartwood was subjected to very high temperatures<sup>[36]</sup>. On the other hand, the amount of oak lactones was also influenced by heartwood grain. The average concentration of oak lactone was significantly decreased ( $P < 0.05$ ) from 162  $\mu\text{g/g}$  in fine-grained heartwoods to 40  $\mu\text{g/g}$  in coarse samples. As is known, the oak lactones have aromas described as oaky, coconut, or vanilla at a low concentration<sup>[37]</sup>. Moreover, it has also been reported that the *cis*-oak lactone has a more powerful aroma than the *trans*-isomer with reported threshold values being 74 and 320  $\mu\text{g/L}$  for *cis*- and *trans*-isomers, respectively<sup>[34]</sup>. In general, the oak lactone isomer ratio range between 1 and 5 in French oak<sup>[38-39]</sup>. In our samples, the *cis*-oak lactone was detected at a higher concentration than the *trans*-counterpart (31.0–125.8  $\mu\text{g/L}$

of *cis*-isomer and 8.7–35.7  $\mu\text{g/L}$  of *trans*-isomer from coarse to fine-grained oak samples, respectively), which were similar to those showed in early papers in wines aged in oak barrels or with other oak products<sup>[38-39]</sup>. This suggested that it is possible to distinguish oaks of different grain types on the basis of their content and ratio of *cis*- and *trans*-oak lactones.





A. *cis*-Oak lactone; B. *trans*-Oak lactone; C. Eugenol; D. Phenol; E. *o*-Guaiacol; F. 4-Vinylguaiacol; G. *cis*-Isoeugenol; H. *trans*-Isoeugenol; I. Syringol; J. Furfural; K. HMF; L. 5-Methyl-furfural.  
**Fig. 2** Evolution of concentrations of volatile compounds in toasted oak heartwoods with different grains

Phenol, *o*-guaiacol, 4-vinylguaiacol, and syringol as well as eugenol and isoeugenol are the main volatile phenols with smoky and clove aroma characteristics. These compounds originate from the thermo-degradation of lignins<sup>[40]</sup> and, except for phenol and eugenol, showed the highest concentration in the medium toasted samples for all the three grain types. Fig. 2C shows the change in eugenol concentration in the different grained oaks with light, medium, and heavy toasting. For all toasting treatments, the fine-grained oak had the highest levels of eugenol and the levels were steadily decreased as the toasting increased. This decrease with severity of heat treatment is similar to previous reports<sup>[41]</sup>. This indicates that the rearrangement effects may lead to the formation of isoeugenol in the isomerization reaction during toasting. Phenol normally has a pungent aroma when its concentration is above 6 000 µg/L<sup>[42-43]</sup>. The phenol levels for all grain types were increase with increasing levels of toasting (Fig. 2D), showing significantly higher concentrations in the heavy-toasted oak heartwoods. The *o*-guaiacol content of all grain types was increased with the initial light toasting but thereafter all was decreased with increasing toasting. The fine-grained oak samples decreased significantly from the light to the heavy toasting intensity, with the average concentration being decreased by about 32% in the medium toast and 44% in the heavy toast. However these were still higher than in the untoasted sample (Fig. 2E). In a similar manner, the toasted of medium- and coarse-grained samples also lost 25% and 38%, and 22% and 41%, for the medium and heavy toasting regimes respectively. Both light and medium toasting of the heartwood increased the concentration of 4-vinylguaiacol. Its concentration ranged from 0.5 µg/g (coarse-grained untoasted heartwood) to 8.3 µg/g (medium-toasted fine-grained heartwood) (Fig. 2F). However, further toasting resulted in a decrease in all three grain types. The presence of the two isomers of isoeugenol was also important because the odor characteristics of isoeugenols are usually described as floral and clove<sup>[44]</sup>. As shown in Fig. 2G and Fig. 2H, the heartwood samples exhibited a similar profile with heating for the two isoeugenol isomers. The isoeugenol concentration was higher in the fine-grained heartwood than in the other heartwoods ( $P < 0.05$ ). Syringol, an important oak heartwood sensory compound, was present in the untoasted heartwoods, but its levels changed with the heat treatment. Similar to isoeugenol, all three grain types showed a maximum with medium toasting but then was dropped with further toasting.

The degradation of polysaccharides produced furanic



aldehydes, mainly being furfural, 5-methylfurfural, and 5-hydroxymethylfurfural (HMF). It is known that degradation of glucose in cellulose releases 5-methylfurfural and HMF, whereas the degradation of pentoses, the principal constituents of hemicelluloses, produces furfural<sup>[4]</sup>. As with previous reports<sup>[45]</sup>, untoasted heartwood was found to contain furfural, 5-methylfurfural, and HMF. It is readily apparent that heat treatment resulted in significant differences in furanic aldehydes (Fig. 2J). The average concentrations of furfural was first increased significantly with light toasting, particularly in the fine-grained oak samples, and then decreased at a higher temperature. This is due to the higher instability of hemicelluloses with temperature. A similar trend was also observed for HMF. The concentration of HMF was increased rapidly with light heat treatment and reached a maximum level at the light toasting level (244 µg/g, 194 µg/g and 166 µg/g from the fine to coarse grains, respectively), as shown in Fig. 2K. In contrast to HMF, the level of 5-methylfurfural was higher for all fine-grained samples than for the medium- and coarse-grained counterparts with corresponding heat treatments (Fig. 2L).

#### 2.4 Analysis of variance

A summary of the analysis of variance (ANOVA) is given in Table 2. The data obtained for the heartwood grain, toasting level, and heartwood grain × toasting level were evaluated by ANOVA. Comparing toasting level, regardless heartwood grain, all the studied compounds showed statistically significant differences related to toasting conditions. It had highlighted the *F*-values of most of LMW phenolics, eugenol, phenol, 4-vinylguaiacol, *trans*-isoeugenol and syringol, all of them are related to polyphenolic characteristics of heartwoods. For example, the oak samples at the heavy toasting level released the highest concentrations of ellagic acid, sinapaldehyde, coniferaldehyde, vanillin, syringaldehyde, vanillic acid, syringic acid and phenol, while the medium toasting level released the highest concentrations of *trans*-isoeugenol, 4-vinylguaiacol and syringol, allowed us to differentiate the samples according to toasting level. These findings agreed with previous studies that had been suggested that the toasted oak heartwoods contained the highest concentration of the oak-derived volatile aroma compounds<sup>[46]</sup>. And the results also indicated that the effect of toasting regimes used might be more important for the oak compounds than for the grain type, because toasting changed the chemical compositions of the heartwood and increased the amounts of several key soluble and volatile compounds as a result of various heat-related reactions. However, ANOVA

also revealed that heartwood grain was significant for certain compounds such as oak lactones, furanic aldehydes, and several volatile compounds, indicating that heartwood grain type affects the formation of these compounds. Moreover, when ANOVA was applied to these data the following two factors need to be taken into account: heartwood grain and toasting level, the higher *F*-values were obtained for *cis/trans*-oak-lactone, *cis*-isoeugenol, furfural, 5-methyl-furfural, total phenol and tannin all related to heartwood grain, followed by ellagic acid, gallic acid, sinapaldehyde, vanillin, phenol, syringol and HMF, related to toasting level. It therefore appeared that there were two factors that would cause differences in the phenolic and aroma compositions of wines: the different amounts of certain compounds provided by each heartwood to wine during ageing could help in the classification of wines according to heartwood grain and toasting condition.

**Table 2** *F*-values and *P* from the analysis of variance (ANOVA) of quantitative evaluation of LMW phenolic and volatile compounds in oak heartwood, and odor threshold and sensory descriptor for each compound

Compounds	Grain	Toasting level	Grain × Toasting	Taste/Odor threshold/ (µg/L) <sup>(24,35,45)</sup>	Sensory descriptor
Ellagic acid	245.7*** (c,b,a)	4 613.5*** (d,c,b,a)	13.7***	1 990	Astringent
Gallic acid	98.9*** (c,b,a)	580.7*** (d,c,a,b)	81.5***	44 970	Astringent
Sinapaldehyde	106.7*** (a,b,c)	2 252.9*** (d,c,b,a)	33.1***		
Coniferaldehyde	1.1	245.7*** (d,c,b,a)	0.5		Heartwoody
Vanillin	88.0*** (a,b,c)	1 469.1*** (d,c,b,a)	10.7***	60	Vanilla, candy
Syringaldehyde	54.8*** (a,b,c)	310.2*** (d,c,b,a)	9.9***	25 000	Vanilla
Vanillic acid	26.2*** (a,b,b)	458.0*** (d,c,b,a)	3.1*	52 967	Astringent
Syringic acid	41.4*** (a,b,c)	145.2*** (d,c,b,a)	7.6**	52 121	Astringent
Ferulic acid	28.7*** (b,a,a)	59.7*** (a,b,c,d)	3.6*	13 011	Astringent
<i>cis</i> -Oak-lactone	472.3*** (a,b,c)	235.9*** (c,b,a,c)	78.0***	74	Heartwoody, coconut, vanilla
<i>trans</i> -Oak-lactone	1 515.2*** (a,b,c)	1 263.0*** (d,c,b,a)	245.6***	320	Heartwoody, coconut, vanilla
Eugenol	18.3*** (a,b,c)	33.4*** (a,b,c,d)	1.2	6	Spices, cinnamon, clove
Phenol	68.7*** (a,b,c)	137.9*** (d,c,b,a)	11.3***	5 900	Pungent
<i>o</i> -Guaiacol	154.9*** (a,b,b)	42.3*** (c,a,b,c)	4.4*	10	Medicine, sweet, smoke
4-Vinylguaiacol	112.1*** (a,c,b)	416.2*** (d,c,b,a)	8.1**	40	Spices
<i>cis</i> -Isoeugenol	113.1*** (a,b,c)	71.7*** (d,c,a,b)	10.3***	10	Flower, clove
<i>trans</i> -Isoeugenol	31.8*** (a,b,c)	103.8*** (d,b,a,b)	5.8**	6	Flower, clove
Syringol	388.7*** (a,b,c)	698.3*** (d,b,a,c)	75.6***	570	Phenolic, medicine
Furfural	201.4*** (a,b,c)	35.6*** (b,a,b,c)	31.7***	20 000	Burnt almonds
5-Hydroxymethylfurfural (HMF)	62.5*** (a,b,c)	382.4*** (d,a,b,c)	11.4***	900	Burnt almond
5-Methyl-furfural	286.6*** (a,b,b)	178.1*** (d,a,b,c)	22.7***	16 000	Bitter almond, spice
Total phenol	356.0*** (c,b,a)	310.3*** (d,c,a,b)	69.5***		
Total tannin	1 374.5*** (c,b,a)	95.6*** (a,b,c,d)	10.7***		

Notes: \*, \*\*, and \*\*\* indicate significance at  $P < 0.05$ ,  $P < 0.01$ , and  $P < 0.001$ , respectively. In the column "Grain", letters between parentheses show the significance among grains, in the order fine, medium, and coarse type grain. In the column "Toasting level", letters between parentheses show the significance among them, in the order untoasted, light, medium, and heavy. Different letters denote a statistical difference with 95% confidence level (LSD), with a being the highest concentration.

#### 2.5 Principal component analysis

Principal component analysis (PCA) was performed on

the data listed in Table 3. The first two principal components (PCs) accounted for 77.37% of the total variance, and they were considered worthy of further discussion.

**Table 3** Eigenvalues, percentage of explained and accumulated variance and coefficients of the first two PCs in PCA

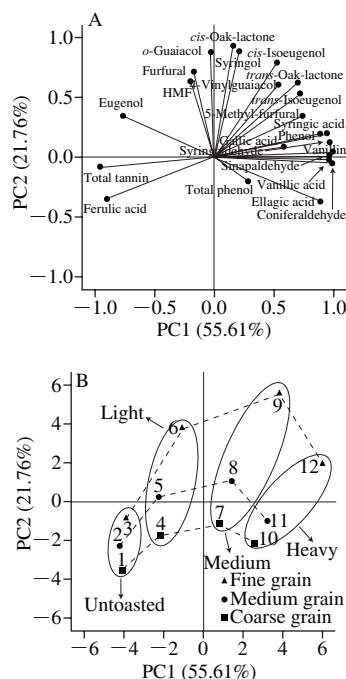
Compounds	PC1	PC2
Eigenvalue	12.790	5.005
Explained variance/%	55.608	21.760
Accumulated variance/%	55.608	77.367
Ellagic acid	0.887	-0.374
Gallic acid	0.579	0.085
Sinapaldehyde	0.959	-0.021
Coniferaldehyde	0.985	-0.050
Vanillin	0.992	0.043
Syringaldehyde	0.965	0.125
Vanillic acid	0.964	0.006
Syringic acid	0.946	0.197
Ferulic acid	-0.900	-0.350
<i>cis</i> -Oak-lactone	0.156	0.928
<i>trans</i> -Oak-lactone	0.697	0.621
Eugenol	-0.765	0.344
Phenol	0.887	0.199
<i>o</i> -Guaiacol	-0.033	0.879
4-Vinylguaiacol	0.539	0.608
<i>cis</i> -Isoeugenol	0.525	0.788
<i>trans</i> -Isoeugenol	0.714	0.531
Syringol	0.206	0.879
Furfural	-0.174	0.711
5-Hydroxymethylfurfural (HMF)	-0.202	0.633
5-Methyl-furfural	0.736	0.346
Total phenol	0.283	-0.204
Total tannin	-0.956	-0.083

Fig. 3A shows the loading plot of PC1 and PC2 derived from the PCA analysis. The variables of the first component (PC1), which accounted for 55.61% of the total variance. The more correlated variables were vanillin, coniferaldehyde, syringaldehyde, vanillic acid, sinapaldehyde, total tannin, syringic acid, and ferulic acid, and to a minor extent, ellagic acid, phenol, eugenol, 5-methylfurfural, and *trans*-isoeugenol. For the second principal component (PC2, 21.76% of the total variance), the strongly correlated attributes were *cis*-oak-lactone, *o*-guaiacol, and syringol, all showing high positive values.

Fig. 3B shows the corresponding scores scatter plot used to establish the relative importance of each component to relate the compounds to each other and with the oak samples. Four well-defined groups could easily be discriminated according to their toasting degrees (untoasted, light, medium, and heavy) along PC1. Toasting effects were well explained by differences in chemical composition of compounds correlated to PC1, namely LMW phenolics, volatiles and other related chemical compounds, which separated the

medium- and heavy-toasted samples on the right hand side of the score plot from the untoasted and light-toasted ones on the left hand side. Consequently samples 8, 9, and 12 (medium toasted medium- and fine-grained samples, and heavy toasted fine-grained ones, respectively) were easily distinguished along PC1 and PC2, which were related mainly to the volatile compounds (*cis/trans*-oak-lactone, syringol, *cis/trans*-isoeugenol, 5-methylfurfural, vanillin, and syringaldehyde). In general, the oak heartwood contains a high level of volatile compounds that would have a great impact on heartwood-matured wine aroma. For example, vanillin concentration in white wines was positively correlated with ‘smoky’ and ‘cinnamon’ descriptors, and in red wines, vanillin was associated with ‘vanilla’, as well as with ‘dark chocolate’ aromas<sup>[36]</sup>. Thus, it indicated that these samples might cause modifications in the final aromatic composition of the wine. While, the heavy toasted medium-grained oak sample (sample number 11) was grouped with medium and heavy toasted coarse-grained oak samples (sample numbers 7 and 10). They were scattered on the positive side of PC1 and negative side of PC2, demonstrating a positive correlation with ellagic acid and total phenol. This may affect the organoleptic characteristics of samples because these compounds are associated with a puckering astringent mouth feel<sup>[47]</sup>, possibly because of the increased depolymerized tannins produced by this toasting regime. On the other side, oak sample numbers 5 (lightly toasted medium-grained) and 6 (lightly toasted fine-grained) had a similar chemical composition and were located in the top left quadrant of the PCA plot, showing a positive correlation with the furanic aldehydes (furfural and HMF) and volatile phenols (eugenol, *o*-guaiacol, and 4-vinylguaiacol). The sensory impact of these components would play an important role in developing the “toasted”, “burnt”, or “caramel” aromas in samples 5 and 6 barrel-aged wines. Finally, the remaining samples (numbers 1–4 represent untoasted coarse-, medium- and fine-grained oak samples, and light toasted coarse grain oak samples, respectively) were located at the negative side of PC1 and PC2, showing a negative correlation with volatiles, and highest levels of total tannin and ferulic acid, particular in sample 1. And because the samples 1–4 demonstrated a good affinity with the total tannin, which probably affected the organoleptic characteristics since it has been shown to be related to bitterness and astringency produced by wine at taste thresholds lower than the concentrations detected in some beverages after it contact with oak heartwood. In addition

to this study, further studies on organoleptic characteristics, including sensory evaluation and GC-olfactometry, should be carried out for a better understanding of the influence that the oak heartwoods can have on the quality of aged wines.



A. Loadings of oak LMW phenolic and volatile compounds on the first two principal components; B. Distribution of oak samples on the plane determined by the first and second components (score plot).

Fig. 3 PCA results

### 3 Conclusions

This study aimed to reveal the differences in the LMW phenolics and volatiles of cooperage oak heartwood of different grain types and the influence of different toasting treatments on the chemical constituents of such heartwoods. According to the our results, the greater the increase in the toasting was, the greater the change in chemical composition of the heartwood extracts was. In particular a higher temperature and time combination during the toasting process increased the degradation of compounds. For example, the oak samples at the heavy toasting level released the highest concentrations of ellagic acid, sinapaldehyde, coniferaldehyde, vanillin, syringaldehyde, vanillic acid, syringic acid and phenol, while the medium toasting level released the highest concentrations of *trans*-isoeugenol, 4-vinylguaiacol and syringol, allowed us to differentiate the samples according to toasting level. Independent of toasting, fine-grained heartwood samples were found

to be higher in volatile compound concentrations, e.g., *cis/trans*-oak-lactone, syringol, *cis/trans*-isoeugenol, furanic aldehydes, and LMW phenolic aldehydes, particularly sinapaldehyde, coniferaldehyde, vanillin, and syringaldehyde. However, medium- and coarse-grained heartwoods were higher in the total tannin, ellagic acid, and gallic acid of LMW phenolic acids. Using principal component analysis, it was possible to discriminate the various heartwood samples studied, and in particular, medium toasted medium- and fine-grained and heavy-toasted oak samples could be distinguished based on the content of volatile compounds (*cis/trans*-oak-lactone, syringol, *cis/trans*-isoeugenol, 5-methylfurfural, vanillin, and syringaldehyde), indicating that these samples may cause modifications in the final aromatic composition of the wine. The heavy toasted medium-grained, and medium and heavy toasted coarse-grained oak samples were related mainly to the ellagic acid and total phenol. These high correlations of ellagic and total phenol could have an effect on the organoleptic characteristics. The lightly toasted fine- and medium-grained oak samples demonstrated a good affinity with the furanic aldehydes (furfural and HMF) and volatile phenols (eugenol, *o*-guaiacol, and 4-vinylguaiacol), which would play an important role in developing the “toasted”, “burnt”, or “caramel” aromas. Moreover, the untoasted and lightly toasted coarse-grained samples were probably related to bitterness and astringency characteristics since they have been shown to be high tannins derived from the heartwood. Of course, further research, including sensory evaluation and GC-olfactometry, should be carried out.

The study should be extended by using a large number of *Q. petraea* oak samples as well as different species and geographical origins of oak; different sizes of oak heartwoods and more detailed toasting process should be investigated. Moreover, the effect of toasting regimes on different species such as chestnut (*Castanea sativa*), ash (*Fraxinus excelsior* and *F. vulgaris*), false acacia (*Robinia pseudoacacia*), and cherry (*Prunus avium*), which have been used for wine barrels, should also be investigated. Thus, the next study will compare the differences in the LMW phenolics and volatile compounds of oak heartwoods of different grain types and in different regions and oak/alternative species during the toasting process.

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