

Influence of the Species and Geographical Location on Volatile Composition of Spanish Oak Wood (*Quercus petraea* Liebl. and *Quercus robur* L.)

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The influence of the species and geographical origin on the volatile composition of wood samples from 80 Spanish oaks (55 *Quercus petraea* Liebl. and 25 *Quercus robur* L.) has been studied. Oak volatile components were isolated by simultaneous distillation–extraction and analyzed by gas chromatography–mass spectrometry. *cis*- and *trans*- β -methyl- γ -octalactones were the main constituents, the *cis* stereoisomer being predominant. Other important volatile components were furfural, 5-methylfurfural, guaiaicol, eugenol, vanillin, or syringaldehyde. The main differences were established between species, *Quercus petraea* being significantly richer in volatile compounds than *Quercus robur*; however, the variability found among trees was high. Differences among geographical provenances were much less important than those found between species.

KEYWORDS: Oak; *Quercus petraea*; *Quercus robur*; geographical location; volatile compounds

INTRODUCTION

Approximately 250 species of the genus *Quercus* exist distributed all over the world. However, only three are used for aging wines: *Quercus petraea* (sessile oak), *Quercus robur* (pedunculate oak), and *Quercus alba* (American oak). Although France is presently the first producer of wood of European oaks (*Q. petraea* and *Q. robur*) for use in wine aging, these two species can also be grown in other zones of Europe, such as Spain, Portugal, Hungary, or Russia.

The chemical composition and sensorial characteristics of wood of French oaks have been widely studied, and a high variability between species, geographic provenance, and individual trees has been found (1–9). Volatile component concentrations are usually greater in wood of *Q. petraea* oak than in *Q. robur* oak (8). Many volatile compounds have been found in oak wood, but only a few of them are significant because of their impact in the sensory characteristic of wines. *cis*- and *trans*- β -methyl- γ -octalactones (oak lactones) have been described as being responsible for the oak flavor and having a low perception threshold (10, 11). Vanillin is the only aldehyde related to lignin that exerts some effect on the aroma of wines aged in oak. Small

free phenols such as eugenol and guaiaicol provide spice and smoke attributes (12).

Oaks from central Europe (Bulgaria and Hungary) and Russia are being used as alternatives to French oaks in the barrel-making industry; however, there is little information about these types of oaks in the literature. Díaz-Maroto et al. (13) studied the volatile composition of Hungarian and Russian oaks by solid-phase microextraction (SPME). However, because high variability between trees of the same species and provenance was found for French and Spanish oaks (7, 8, 14, 15), more studies are necessary to characterize these types of oaks.

At the moment, Spanish oaks are not used commercially at great scale; nevertheless, studies realized on their chemical composition, as well as their behavior during the wine aging process, have made clear their possibilities as an alternative to the traditionally exploited French and American oaks (3, 14–16). Spanish oak forests are spread all over the northern and northwestern regions of the Iberian peninsula. Therefore, to draw conclusions on the potential of the Spanish oaks as alternatives to the oaks traditionally used in the aging of wines, it is necessary to study the chemical composition of a larger sample set of Spanish oaks from different regions.

Variability among trees of the same species or region and differences in the treatments applied to the casks must be taken into account when species are differentiated, and the sampling method employed is of great importance. Towey and Waterhouse (17) reported variabilities of 30% on the chemical

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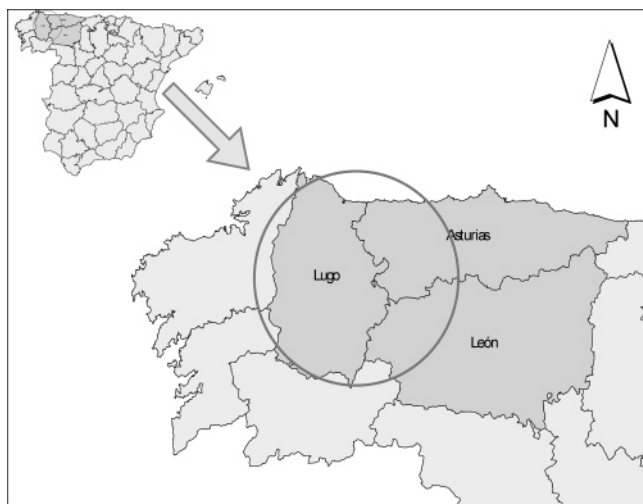


Figure 1. Geographical origins of the Spanish oak wood samples.

composition of oak casks from the same lot when 4 casks per lot were sampled, these dropping to 10% when 39 casks were sampled.

Different methods have been used for the analysis of volatile wood components, most of them based on the extraction with organic solvents directly or previously macerating the wood in aqueous alcoholic solutions (1, 15, 18–20). These methods are time-consuming and in general require large amounts of sample. For a rapid screening of volatile compounds in oak wood, direct thermal desorption (DTD) or SPME can be used (13, 21, 22). Both methods are useful for sample characterization; however, high temperature must be applied to the sample in DTD, and the recovery of the SPME is only partial and depends on the compound.

Simultaneous distillation–extraction (SDE) using dichloromethane as extractive solvent has been found to be advantageous for an effective enrichment of wood extracts in important compounds from a sensorial standpoint (lactones, eugenol, vanillin, or syringaldehyde) using small amounts of sample and extractive solvent (19).

The aim of this study was to investigate the influence of the species and the geographical location on the volatile composition of wood from Spanish oaks, using a set of 80 trees of 2 species (*Quercus petraea* Liebl. and *Quercus robur* L.) from different geographical provenances, which have not been previously studied.

MATERIALS AND METHODS

Samples. The sample set included wood from two species of Spanish oaks, 55 *Q. petraea* and 25 *Q. robur*, from six geographical locations in the northwestern region of the Iberian peninsula, in Spain (Figure 1). Dominant trees in the sampling zones were selected and felled (23, 24). From each tree, disks of wood were obtained at a height of 1.3 m from the base of the trunk. From each disk, test tubes (heartwood) measuring 20 × 20 × 40 mm were obtained following the UNE 56-528-78 standards (25).

The wooden blocks were dried as follows: The blocks were saturated with water, to the green or saturated state, and then stabilized to 12% of internal humidity at 20 ± 2 °C and 65% relative humidity. Finally, the blocks were heated to dryness (0% internal humidity) in an oven at 103 ± 2 °C. Wooden blocks were ground using an iron file.

Isolation of Volatile Components. Volatile compounds were isolated by SDE in a microscale apparatus (Chrompack, Middelburg, The Netherlands) previously described (19, 26). Ten grams of dry wood shavings was put into a 250 mL round-bottom flask with 100 mL of Milli-Q water and 100 μL of a γ-caprolactone solution in absolute

Table 1. Influence of Species on Volatile Composition of Spanish Oak Woods

compound	species			
	<i>Q. petraea</i> (n = 55)		<i>Q. robur</i> (n = 25)	
	mean (μg/g)	range (μg/g)	mean (μg/g)	range (μg/g)
furfural	90.3	22.0–273.3	21.6	4.53–83.4
5-methylfurfural	18.1	2.31–50.7	1.79	0.13–8.90
<i>o</i> -cresol	0.39	0.00–4.98	0.09	0.04–0.24
<i>p</i> -cresol	0.42	0.05–3.58	0.14	0.03–0.71
guaiacol	0.91	0.09–2.62	0.21	0.04–0.76
4-methylguaiacol	2.92	0.29–11.7	0.07	0.01–0.49
4-propylguaiacol	0.20	0.00–1.42	0.06	0.00–1.03
(<i>Z</i>)-2-nonenal	2.52	0.15–6.17	3.28	0.46–8.68
decanal	1.52	0.23–2.83	0.75	0.35–1.28
<i>trans</i> -oak lactone	26.8	0.43–115.1	6.02	0.03–55.7
<i>cis</i> -oak lactone	35.7	0.70–158.6	10.1	0.05–49.6
eugenol	0.75	0.02–2.49	1.14	0.01–4.49
methyleugenol	0.21	0.01–1.20	0.52	0.00–3.19
isoeugenol	0.25	0.00–1.21	0.15	0.02–0.73
vanillin	1.91	0.13–20.1	0.19	0.00–2.16
syringaldehyde	0.73	0.00–9.83	0.17	0.00–1.74

ethanol (1.056 g/L) added as internal standard. The extraction was performed under atmospheric conditions for 2 h using dichloromethane (2 mL) as extraction solvent. The same sample was re-extracted again using fresh solvent, no compounds being found in that second step. Four replications of the extraction and analysis procedure were performed for one wood sample. Values smaller than 15% were obtained for relative standard deviations (RSDs). This method has satisfactorily been used to isolate the volatile components of many vegetal samples (27, 28). The collected extracts (1 mL) were concentrated to 0.5 mL under nitrogen and stored in the freezer until gas chromatographic analysis.

Gas chromatography–mass spectrometry (GC-MS) was carried out on a Hewlett-Packard G1800B GCD system that consists of a gas chromatograph, an electron ionization detector (EID, 70 eV), and a data system. One microliter of dichloromethane extract was injected in splitless mode during 0.1 min (split ratio of 1:20) on an SPB-1 (Supelco) methyl silicone column (50 m × 0.25 mm × 0.25 μm film thickness). The column temperature program was 70 °C (3 min) and then raised at 3 °C/min to 250 °C (5 min). Inlet and transfer line temperatures were 250 and 280 °C, respectively. Mass detector conditions were as follows: source temperature, 178 °C; scanning rate, 1 scan/s; mass acquisition range, 40–450.

Identification of the volatile components was performed by comparing their GC retention indices and mass spectra with those of authentic standards from Sigma-Aldrich. Semiquantitative analysis of the compounds was performed by assuming that component response factors were the same as the response factor for the internal standard. The concentration of each compound was calculated in milligrams per gram of dry weight.

Statistical Analysis. Chemical data were analyzed by using the Student–Newman–Keuls test for comparison of the means (different letters in the same row indicate statistical differences at the 0.05 level according to the Student–Newman–Keuls test) and by cluster analysis and discriminant analysis using the SPSS 11.0 program.

RESULTS AND DISCUSSION

Table 1 shows the most important volatile components found in Spanish oak woods from two species (*Q. petraea* and *Q. robur*) independent of their origin. As has been previously described (7, 8), *Q. petraea* was significantly richer in volatile compounds than *Q. robur*; however, the variability found among trees was high. Other authors have observed that natural seasoned and toasted Spanish oaks from Alava did not show significant differences among species and provenance (14, 15).

Table 2. Tests of Equality of Group Means

compound	Wilks' lambda	F	signif
furfural	0.689	35.134	0.000
5-methylfurfural	0.606	50.813	0.000
<i>o</i> -cresol	0.952	3.971	0.050
<i>p</i> -cresol	0.932	5.686	0.020
guaiacol	0.962	3.085	0.083
4-methylguaiacol	0.732	28.543	0.000
4-propylguaiacol	0.950	4.077	0.047
(<i>Z</i>)-2-nonenal	0.959	3.366	0.070
decanal	0.703	32.875	0.000
<i>trans</i> -oak lactone	0.886	10.056	0.002
<i>cis</i> -oak lactone	0.903	8.411	0.005
eugenol	0.946	4.447	0.038
methyleugenol	0.931	5.765	0.019
isoeugenol	0.965	2.802	0.098
vanillin	0.901	8.564	0.004
syringaldehyde	0.964	2.950	0.090

To determine which compounds are more characteristic of *Q. petraea* and *Q. robur*, discriminant analysis was applied to the quantitative data obtained for the samples grouped according to these species. The variables that contributed to this differentiation were those with the smaller Wilks's lambda (Table 2). Wilks's lambda was significant by the *F* test for 5-methylfurfural, furfural, decanal, and 4-methylguaiacol. The percentages of correct classification were 94.5 and 92.7% by cross-validation for *Q. petraea* and 92.0 and 80.0% by cross-validation for *Q. robur*.

After furfural, *cis*- and *trans*- β -methyl- γ -octalactones (oak lactones) were the main constituents in all of the samples, the *cis*-stereoisomer being predominant (17, 20, 21, 29). However, in some cases, the *trans*-stereoisomer was found at higher concentrations than the *cis*, particularly in *Q. robur* species. The *cis/trans* ratio is a useful index to differentiate among types of oak wood (21, 30, 31); however, in our case this dependency is not clear due to the high variability found among trees.

In the case of volatile compounds related to lignin, such as vanillin and syringaldehyde, higher differences between species

were found for vanillin, although, as for oak lactones, the variability among trees was quite great. Other volatile compounds found in greater concentrations in *Q. petraea* were guaiacol and its derivatives (4-methylguaiacol and 4-propylguaiacol), cresols, furfural, 5-methylfurfural, and decanal. Furfural and 5-methylfurfural appeared in all of the samples. Both compounds can increase after seasoning, especially when high temperatures are used (32). In our study, samples were stabilized to the same water content under the same conditions. For this reason, despite the high variability among trees, *Q. petraea* trees had higher quantities of furfural and 5-methylfurfural than *Q. robur*. (*E*)-2-Nonenal and decanal are associated with the "sawdust" character of untreated oak wood (33), and their concentrations decrease with the toasting of barrels.

In general, *Q. petraea* trees are higher in extractable volatile compounds than *Q. robur* trees (12, 34); however, Spanish oak woods did not show significant differences, revealing variations among production zones and individual trees that have been previously established (8, 15, 35).

The volatile compositions of Spanish oak woods of different geographic locations together with the results of the Student–Newman–Keuls test for comparison of the means, are given in Table 3. It can be observed that the variability between species was higher than the variability found among samples of different geographical locations. The main compounds that contributed to this differentiation were 5-methylfurfural, 4-methylguaiacol, and decanal. In the case of the oak lactones, a clear influence of the geographical location was observed. Samples with greatest concentrations of both stereoisomers were of *Q. petraea* species from Galicia, followed by those from Asturias. *Q. petraea* from Leon presented oak lactone contents similar to those of *Q. robur* species. It is worth noting the low concentration of oak lactones of *Q. robur* species from northern Lugo, although due to the high variability among trees, the differences were not significant. Other oak woods with small amounts of oak lactones are those from Hungary and Russia, although at the moment there is little information about the volatile composition of these oak woods (13). Although no

Table 3. Volatile Composition of Spanish Oak Wood of Different Species and Geographic Location

compound	geographic location ^a											
	<i>Q. petraea</i>						<i>Q. robur</i>					
	Asturias (QpAST) (n = 22)		Galicia (QpGAL) (n = 16)		Leon (QpLEO) (n = 17)		northwestern Lugo (QrNWL) (n = 10)		northern Lugo (QrNL) (n = 8)		rest of Galicia ^b (QrRG) (n = 7)	
mean (μ g/g)	range (μ g/g)	mean (μ g/g)	range (μ g/g)	mean (μ g/g)	range (μ g/g)	mean (μ g/g)	range (μ g/g)	mean (μ g/g)	range (μ g/g)	mean (μ g/g)	range (μ g/g)	
furfural	62.9ac	22.0–179.1	129.5b	42.7–273.3	89.1c	29.5–257.5	23.6ad	5.30–81.3	30.9ad	4.53–83.4	8.15d	2.75–20.8
5-methylfurfural	15.4a	6.37–47.1	21.6a	2.31–50.7	18.2a	2.92–35.3	1.81b	0.36–7.44	2.73b	0.71–8.90	0.70b	0.13–2.37
<i>o</i> -cresol	0.18a	0.00–0.76	0.84b	0.03–4.98	0.26a	0.05–0.76	0.09a	0.05–0.20	0.10a	0.04–0.24	0.07a	0.04–0.14
<i>p</i> -cresol	0.40a	0.05–2.62	0.54a	0.05–3.58	0.35a	0.10–0.87	0.18a	0.04–0.71	0.09a	0.03–0.19	0.13a	0.07–0.23
guaiacol	0.60a	0.14–1.80	1.28a	0.23–2.62	0.96a	0.09–2.59	0.15a	0.07–0.33	0.09a	0.04–0.16	0.42a	0.05–0.76
4-methylguaiacol	2.70a	0.29–11.7	3.14a	0.56–6.76	3.01a	0.38–9.33	0.05b	0.01–0.15	0.13b	0.01–0.49	0.04b	0.02–0.13
4-propylguaiacol	0.15a	0.00–0.92	0.29a	0.01–1.42	0.19a	0.01–1.35	0.01a	0.00–0.02	0.18a	0.01–1.03	0.01a	0.00–0.02
(<i>Z</i>)-2-nonenal	2.37a	0.15–5.90	3.30a	0.72–6.17	1.23a	0.68–4.16	3.30a	1.70–6.45	3.26a	0.70–8.68	3.26a	0.46–7.71
decanal	1.47a	0.77–2.41	1.97b	0.56–2.83	1.15a	0.23–2.15	0.62c	0.35–1.12	0.86c	0.33–1.28	0.81c	0.53–1.21
<i>trans</i> -oak lactone	24.1ab	0.60–71.8	50.5a	0.43–115.1	8.02b	0.54–34.6	3.09b	0.10–15.2	0.14b	0.03–0.44	16.9b	0.17–55.7
<i>cis</i> -oak lactone	36.8ab	0.82–149.8	59.5a	0.70–158.6	11.8b	1.13–45.8	8.67b	0.13–40.8	0.29b	0.05–0.78	23.2b	0.26–49.6
eugenol	0.65a	0.05–1.49	0.89a	0.02–2.27	0.77a	0.05–2.49	1.38a	0.19–3.30	0.67a	0.01–2.25	1.34a	0.18–4.49
methyleugenol	0.20a	0.01–0.69	0.24a	0.02–1.20	0.21a	0.01–1.10	0.72a	0.00–2.60	0.57a	0.01–3.19	0.16a	0.00–0.77
isoeugenol	0.22a	0.03–1.20	0.24a	0.04–1.21	0.29a	0.00–1.20	0.07a	0.02–0.17	0.32a	0.05–0.73	0.06a	0.03–0.11
vanillin	2.34a	0.25–20.1	1.86a	0.14–7.18	1.40a	0.13–6.89	0.08a	0.00–0.26	0.41a	0.07–2.16	0.10a	0.01–0.29
syringaldehyde	0.89a	0.00–9.83	0.80a	0.00–4.45	0.46a	0.00–4.99	0.03a	0.00–0.22	0.42a	0.00–1.74	0.07a	0.01–0.19

^a Different letters in the same row indicate statistical differences at the 0.05 level according to the Student–Newman–Keuls test. ^b Corresponds to the rest of the Galician territory with the exception of the northern zone and northwestern Lugo).

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