

Chemical Characterization of Oak Heartwood from Spanish Forests of *Quercus pyrenaica* (Wild.). Ellagitannins, Low Molecular Weight Phenolic, and Volatile Compounds

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The need for new sources of quality wood supply for cooperage has led to looking into the possibility of utilizing *Quercus pyrenaica* Wild. oak, a species native to the Iberian peninsula, as an alternative to other European (*Quercus robur* and *Quercus petraea*) and American (*Quercus alba*) oaks. The low molecular weight phenolic composition, ellagitannins, and volatile compounds (including a wide range of compound families such as volatile phenols, furanic compounds, lactones, phenyl ketones, other lignin-derived compounds, and volatile compounds related to off-flavors) of green heartwood from Spanish forest regions were studied by HPLC and GC, in order to know its enological characteristics. The chemical composition of *Q. pyrenaica* is similar to that of other species commonly used in cooperage to make barrels, showing only quantitative differences that were more significant with respect to American than to French species. The four provenance regions studied showed similar chemical composition, with high variability among individuals, often higher than the variability among regions of provenance, but in line with that described in other European and American oak woods. Therefore, this species must be considered to be suitable for aging wine.

KEYWORDS: *Quercus pyrenaica*; oak wood; polyphenols; ellagitannins; volatile compounds

INTRODUCTION

The need for new sources of quality wood supply for cooperage has led to looking into the possibility of utilizing Spanish oak as an alternative to French (*Quercus robur* and *Quercus petraea*) and American (*Quercus alba*) oaks, which are the most commonly used in enology. According to the last Spanish forest inventory (1999) (1), in Spain, in addition to forests of *Q. robur* and *Q. petraea*, forests of another species of *Quercus*, *Quercus pyrenaica* Wild., can be found. This *Quercus* species is native to the Iberian peninsula, where it is known as *rebollo* or *melejo*. Its forest cover area in Spain is approximately 320 000 ha, distributed mostly in the northwestern quadrant.

Traditionally, this wood has been used in Spain for railway sleepers and wooden ships and, in recent years especially, as firewood of low forest, an arboreal mass composed of feet coming from buds or roots. This has resulted in a progressive degradation of the characteristics of some of these forest areas,

such as a high percentage of trees with a diameter of <40 cm, knotty, twisted, or short-boled trees. Such changes decrease the final proportion of material usable as quality wood. However, studies in recent years have revealed that the chemical composition (polyphenols, tannins, and volatile compounds with an enological interest) of this wood from northern Spain has turned out to be similar to that of *Q. robur* and *Q. petraea* woods, of both French and Spanish origin (2–7). Its structural properties (mesh, grain, density, and permeability) were also appropriate for enological use. Moreover, its suitability was demonstrated with a Rioja Alavesa wine, aged in barrels made of wood from said species, the quality, sensorial (color, gustative, olfactory, and visual perception), and chemical characteristics (polyphenols and volatile compounds related to wood) of which were similar to those obtained from wine aged in barrels made of French oak wood, renowned in the wine-aging process (8, 9).

As a result, we developed an important plan for a functional silviculture project to obtain quality wood for barrel staves from different Spanish regions. Knowledge of the chemical composition of oak wood is the first step in predicting which components should be expected in the final product after aging. In this first step, it is necessary to study green wood, to compare its main characteristics with those of wood commonly used in cooperage. From an enological point of view, the main extractable oak wood

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components are polyphenols, ellagitannins, and volatile compounds. During the aging of wines and spirits in oak barrels, a progressive solubilization of ellagitannins occurs, and oxidizing processes slowly transform these molecules into polymerized and colored polyphenols (10). They then contribute to the color of wines and spirits stored in barrels. It has been suggested that they may also increase the astringency and bitterness of wines aged in oak barrels (11) and affect the solubility of the other oak compounds, such as volatile compounds (12). The ellagitannins also intervene in redox reactions, which modify the structure of tannins and the color substances of red wines (10). The main volatile compounds susceptible to migration from oak wood to wine are the *cis* and *trans* isomers of β -methyl- γ -octalactone, furfural and its derived compounds, phenolic aldehydes such as vanillin and syringaldehyde, and volatile phenols such as eugenol, guaiacol, and ethyl- and vinylphenols. Phenolic aldehydes such as vanillin contribute to olfactory characteristics of wines with notes of vanilla, coffee, black chocolate, and smoke, with a synergistic effect of whiskey lactone (13, 14). Their effect can be reduced because during aging a biological degradation causes the formation of the corresponding alcohol and ethyl ether (14). Finally, several pigments were recently found to be produced from the reaction between catechin and cinnamic aldehydes, such as coniferyl or sinapyl, extracted from oak wood (15). The structure and concentration of oak wood components depend on the species and other factors such as geographical origin and silvicultural treatments. Therefore, these factors are important variables to be considered by the cooper and the winemaker when selecting wood for cooperage. In general, American oak wood is richer than European in low molecular weight phenolic compounds, β -methyl- γ -octalactone, and some volatile compounds, but poorer in ellagitannins. Among European oak woods, *Q. robur* is richer in ellagitannins and poorer in whiskey lactones than *Q. petraea*. However, the variations among individuals from the same species and from the same forest are also important. In the same tree, height, positioning, and age of wood are other possible variation factors in the composition of its oak wood. Although the concentrations are sensitive to cooperage operations such as seasoning and toasting, species discrimination remains statistically significant because each species retains its own chemical criteria of aromaticity and tannicity (8, 9, 16).

In the literature, scant data about the chemical composition of wood from *Q. pyrenaica* have been found, and only the studies carried out by our research group were on green wood (2, 3). Here we report an analysis of low molecular weight phenolic compounds, ellagitannins, and volatile compounds in green oak heartwood from four Spanish forests of *Q. pyrenaica* Wild. in order to learn about its similarity in characteristics with respect to wood commonly used in cooperage.

MATERIALS AND METHODS

Collection of Wood Samples. In 1998, Jiménez-Sancho et al. (17) defined 16 provenance regions of *Q. pyrenaica* in Spain, 10 of them with large forest areas. The samples of this work come from four provenance regions: region 4, southern Cantábrica mountain range; region 6, northern area of Sistema ibérico; region 8, Gata and Peña de Francia; and region 11, north of Sierra de Guadarrama. Selected trees in the sampling zones were felled. The number of trees felled in each region was 14, 7, 18, and 15, respectively. From each tree, wood slices were obtained at a height of 1.30 m from the base of the trunk, separating the heartwood from each slice. The heartwood pieces were ground and sieved, and the sawdust ranging in size from 0.28 to 0.80 mm was taken for analysis.

Standards. Reference compounds were purchased from Fluka Chimie AG (Buchs, Switzerland) (gallic acid, scopoletin, 1-hexanol,

furanyl-1-ethanone, benzaldehyde, butyrolactone, furfuryl alcohol, 2-phenylethanol, benzothiazole, 1*H*-pyrrole-2-carboxaldehyde, 2-phenoxyethanol, syringol, and isoeugenol), Aldrich Chimie (Neu-Ulm, Germany) (vanillic acid, syringaldehyde, coniferyl aldehyde, *trans*-2-nonenal, 1-nonanal, *trans*-2-octenal, *trans*- β -methyl- γ -octalactone, *cis*- β -methyl- γ -octalactone, 4-methylguaiacol, maltol, 4-methylsyringol, and 4-allylsyringol), Apin (ellagic acid), Chem Service (West Chester, PA) (syringic acid, 2-ethyl-1-hexanol, and phenylmethanol), Sigma Chemical (St. Louis, MO) (vanillin, 1-hexanol, furfural, 5-methylfurfural, guaiacol, phenol, eugenol, and 5-hydroxymethylfurfural), and Extrasynthèse (Genay, France) (sinapaldehyde, acetovanillone, acetosyringone, and γ -hexalactone). Standards of vescalagin, castalagin, roburins A and E, and grandinin were kindly provided by Dr. Scalbert.

Extraction of Phenolic Compounds. The sawdust samples (1 g) were extracted with 100 mL of methanol/water (1:1) at room temperature and in darkness for 24 h, following the method described by Fernández de Simón et al. (2). After removal of the methanol in a rotary evaporator at a temperature below 40 °C, the aqueous solution was extracted with diethyl ether and ethyl acetate and then freeze-dried. The diethyl ether and ethyl acetate extracts were used for the HPLC quantitative determination of low molecular weight phenolic compounds, and the freeze-dried extract was used for that of ellagitannins.

Extraction of Volatile Compounds. Volatile compounds were extracted using the following method, based on that described by Chatonnet et al. (18). The sawdust samples (2 g) were soaked in 100 mL of hydroalcoholic solution (12% ethanol, 0.7 g/L tartaric acid, and 1.11 g/L potassium bitartrate) for 15 days at room temperature and in darkness. After filtration of the mixture, we added 100 μ L of internal standard (γ -hexalactone 2 mg/mL in ethanol) and 15 g of ammonium sulfate, and the solution was extracted with 45 mL of dichloromethane, distributed in three times. The organic fraction was dried using anhydrous sodium sulfate and concentrated to 0.5 mL under nitrogen flux in a Kuderna–Danish apparatus before being submitted to GC-MS analysis. In the analysis carried out in duplicate from the same sample the coefficients of variation were <5%.

HPLC-DAD Analysis. An apparatus equipped with a diode array detector and with a C18 Hypersil ODS (5 μ m) column (20 cm \times 4 mm i.d.), protected by a precolumn of the same material, was used. With the diethyl ether and ethyl acetate extract, HPLC analysis of low molecular weight phenolic compounds was carried out according to the method of Cadahía et al. (5). With material from the freeze-dried extract, HPLC analysis of ellagitannins was carried out according to the method of Cadahía et al. (6). Quantitative determinations were carried out by using the external standard method. Roburins B–D were expressed as roburin A equivalents because they are also dimers.

GC-MS Analysis. GC-MS analysis of extracts was carried out according to method of Chatonnet et al. (18). An HP 5890 gas chromatograph (Palo Alto, CA), equipped with an HP 5971A selective mass detector and a fused silica capillary column (Supelcowax-10, 30 m \times 0.25 mm i.d., and 0.25 μ m film thickness) was used, under the working conditions described in Cadahía et al. (7). Chromatographic peaks were identified by comparing their retention times and mass spectra with those of standards and/or reported in literature. Quantitative determinations were carried out by the internal standard method, using peak areas obtained from total ion current (TIC) or selected ion monitoring (SIM), depending of peak purity. The selected ions *m/z* for the last ones are shown in **Tables 3–6**. Calibration was done with a standard or with the closest chemical structure, analyzed under the same conditions.

Statistical Analysis. Chemical data were analyzed by ANOVA and multivariate canonical discriminant analysis, using the SAS program (19).

RESULTS AND DISCUSSION

Low Molecular Weight Phenolic Compounds. The identified compounds, the HPLC quantitative evaluation of which is shown in **Table 1**, were the same phenolic compounds previously found in other *Quercus* species, whether of Spanish, French, or American origin (2). The most abundant component was ellagic acid, followed by gallic acid. Aldehyde contents

Table 1. HPLC Quantitative Evaluation of Low Molecular Weight Phenolic Compounds (Micrograms per Gram of Wood) in Green Oak Wood from *Q. pyrenaica* of Different Regions of Provenance in Spain^a

compound	region of provenance			
	Gata/Peña de Francia	Guadarrama	Cantábrica mountain range	Sistema Ibérico
gallic acid	180 ± 150a	112 ± 92a	143 ± 99.7a	99.5 ± 109a
vanillic acid	5.00 ± 2.26a	4.33 ± 1.75a	3.88 ± 1.46a	3.30 ± 1.38a
syringic acid	6.50 ± 2.48a	5.01 ± 1.57a	7.39 ± 4.85a	4.93 ± 3.11a
ellagic acid	696 ± 193a	626 ± 158a	877 ± 396a	890 ± 215a
vanillin	5.87 ± 1.86a	7.22 ± 2.93a	5.63 ± 1.78a	3.61 ± 2.20b
syringaldehyde	8.94 ± 3.15a	8.52 ± 2.71a	10.32 ± 3.97a	7.27 ± 4.43a
coniferyl aldehyde	4.07 ± 2.11a	3.90 ± 0.91a	3.84 ± 2.17a	2.34 ± 1.33a
sinapic aldehyde	4.74 ± 2.11a	4.24 ± 1.17a	3.86 ± 1.29a	3.20 ± 0.90a
scopoletin	1.08 ± 0.61b	4.42 ± 4.06a	5.61 ± 3.77a	4.23 ± 3.52a

^a Average and standard deviation ($\bar{x} \pm \text{sd}$) were calculated for the number of samples available of each region of provenance. Different letters in a row denote a significant difference with 95% confidence level in the Student–Newman–Keuls multiple-range test.

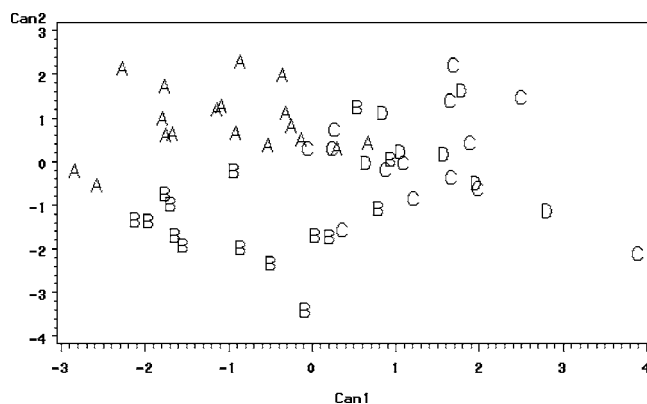


Figure 1. Canonical discriminant analysis of low molecular weight phenolic compounds in green wood of *Q. pyrenaica*, from different regions of provenance in Spain: A, Gata/Peña de Francia; B, Guadarrama; C, Cantábrica mountain range; D, Sistema Ibérico (93.42% of dispersion; canonical correlations of 0.76 and 0.66 and eigenvalues of 1.42 and 0.79, for Can 1 and Can 2, respectively). The total canonical structure coefficients of functions 1 and 2 were, respectively, as follows: gallic acid, -0.15 and 0.31 ; vanillic acid, -0.37 and 0.15 ; syringic acid, 0.11 and 0.27 ; ellagic acid, 0.49 and 0.22 ; vanillin, -0.37 and -0.40 ; syringaldehyde, 0.08 and 0.06 ; coniferylaldehyde, -0.24 and 0.01 ; sinapic aldehyde, -0.37 and 0.12 ; and scopoletin, 0.50 and -0.49 .

were always higher than those of respective acids and benzoic compounds higher than those of cinnamic ones. This was to be expected, taking into account the structure of European oak lignin (20). The concentrations of the majority of these compounds showed a high dispersion, with high standard deviations related to average. This could be explained because the polyphenolic composition varies significantly among individuals from the same forest, and this would result in the few statistically significant differences found among regions of provenance.

To compare the whole polyphenolic composition of *Q. pyrenaica* green woods from different provenance regions, we carried out a canonical discriminant analysis on the compounds evaluated in **Table 1**. This led us to a mathematical model that explained 100% of the dispersion, distributed among five canonical functions. **Figure 1** shows the graphical representation of sample projections of each group on the plane defined by the two principal canonical axes (functions 1 and 2). This represents 93.42% of the dispersion, with canonical correlations of 0.76 and 0.66 and eigenvalues of 1.42 and 0.79, respectively. Discriminant function 1 corresponded mainly to levels of vanillin and ellagic acid, and function 2 corresponded to those of gallic and vanillic acids, according to the coefficients of total

canonical structure included in **Figure 1**. The canonical representation showed a distribution of samples in which no well-defined groups can be distinguished. This indicates that the green woods of *Q. pyrenaica*, from the different regions studied, have highly similar phenolic compositions to each other, showing at the same time high variability among individuals from the same forest.

Similar single-tree effects have been described in other *Quercus* species from northern Spain, France, and America. In general, the concentrations found in this work were similar to those cited by Chatonnet (21) and Fernández de Simón et al. (2), lower than those cited by Miller et al. (22) and Marco et al. (23), and higher than those cited by Nabeta et al. (24) and Mosedale et al. (25), in different geographical origins and species used in cooperage. This was to be expected as the polyphenolic composition of the wood of *Q. pyrenaica* is more similar to that of European oak wood than to that of American oak wood.

Ellagitannins. The HPLC analysis of the aqueous solution from wood samples revealed the presence of the eight ellagitannins so far isolated and identified in other oak woods accepted for enological use (26, 27). **Table 2** shows the quantitative evaluation in woods from the different studied provenances, where roburin A and roburin B are evaluated together because the separation of their chromatographic peaks was not always clear. The most abundant ellagitannins were the monomers, castalagin, roburin E, vescalagin, and grandinin, in this order, with the exception of region 8, in which the average concentration of grandinin was higher than that of vescalagin. In the four regions, their average concentrations were similar, with high standard deviations and consequently insignificant statistical differences, as can be deduced from the variance analysis.

The discriminant analysis carried out on all of the ellagitannins shows a pattern of sample distribution on the plane defined by the two principal canonical axes (**Figure 2**) similar to that of phenolic compounds, where the sets of points from different regions overlap and groups cannot be clearly distinguished.

Comparing these ellagitannin results with those obtained previously in green Spanish, French, and American oak woods by us and other authors, no differences are observed in average concentrations. The wood of *Q. pyrenaica* from studied regions showed ellagitannin concentrations between those of French *Q. robur* (showing the highest values) and American *Q. alba* (showing the lowest values) and similar to those of French *Q. petraea* (3, 28–30). The high variability observed due to the single-tree effect has also been described in other European and American oaks (3, 28–30), and it can be even higher than the variability among species (31).

Table 2. HPLC Quantitative Evaluation of Ellagitannins (Milligrams per Gram of Wood) in Green Oak Wood from *Q. pyrenaica* of Different Regions of Provenance in Spain^a

compound	region of provenance			
	Gata/Peña de Francia	Guadarrama	Cantábrica mountain range	Sistema Ibérico
monomers				
castalagin	10.63 ± 5.11a	11.25 ± 4.24a	12.58 ± 7.37a	11.49 ± 8.93a
vescalagin	3.72 ± 1.90a	5.48 ± 2.72a	5.48 ± 3.37a	4.48 ± 4.49a
pentosylated monomers				
roburin E	5.75 ± 2.44ab	5.50 ± 1.68ab	6.51 ± 3.47a	5.74 ± 3.80ab
grandinin	5.39 ± 1.82a	5.11 ± 1.24a	4.53 ± 1.82a	4.11 ± 2.43a
dimers				
roburin A + B	1.55 ± 0.83a	1.48 ± 0.52a	1.30 ± 0.54a	1.11 ± 0.97a
pentosylated dimers				
roburin D	2.09 ± 0.59a	2.09 ± 0.47a	2.10 ± 0.99a	1.83 ± 0.63a
roburin C	0.40 ± 0.20a	0.17 ± 0.11b	0.22 ± 0.147b	0.32 ± 0.21ab

^a Average and standard deviation ($x \pm sd$) were calculated for the number of samples available of each region of provenance. Different letters in a row denote a significant difference with 95% confidence level in the Student–Newman–Keuls multiple-range test.

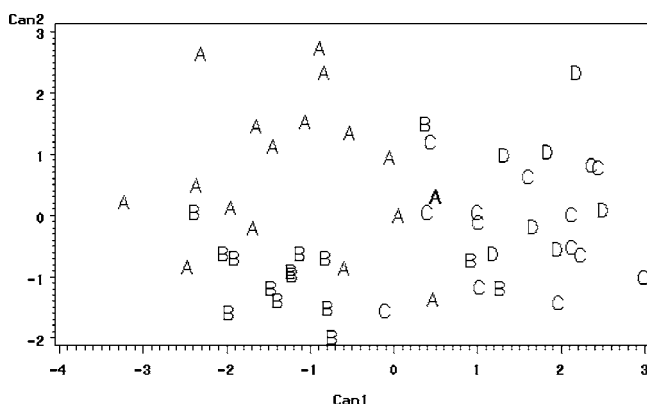


Figure 2. Canonical discriminant analysis of ellagitannins in green wood of *Q. pyrenaica*, from different regions of provenance in Spain: A, Gata/Peña de Francia; B, Guadarrama; C, Cantábrica mountain range; D, Sistema Ibérico (97.92% of dispersion; canonical correlations of 0.80 and 0.54 and eigenvalues of 1.83 and 0.42 for Can 1 and Can 2, respectively). The total canonical structure coefficients of functions 1 and 2 were, respectively, as follows: castalagin, 0.13 and -0.10 ; vescalagin, 0.13 and -0.46 ; roburin E, 0.13 and 0.02; grandinin, -0.31 and 0.06; roburin A + B, -0.25 and 0.02; roburin D, -0.07 and -0.07 ; and roburin C, -0.14 and 0.91.

Volatile Compounds. The GC-MS analysis of hydroalcoholic wood extracts revealed the presence, in fresh Spanish *Q. pyrenaica* oak wood, of the majority of volatile compounds previously found in other Spanish, French, or American oak woods, as much fresh as seasoned or toasted (7, 32–35). The

results of their quantitative evaluations are included in **Tables 3–6** and then studied by arranging them by compound family.

Volatile Phenols. The most abundant component in wood of all provenance regions was eugenol, followed by 4-allylsyringol (**Table 3**), which show concentrations significantly higher than the other ones. Comparing the results obtained from different regions, the only significant differences, according to the Newman–Keuls multiple-range test, were shown by region 8 with respect to the others, which is characterized by higher levels of eugenol, isoeugenol, syringol, 4-methylsyringol, and 4-allylsyringol.

Regarding the canonical discriminant analysis (**Figure 3**) carried out on all of the volatile phenols, the position of samples from Gata/Sierra de Francia (A) with respect to the other regions on the plane defined by two principal canonical axes is statistically more distant. This region is easily distinguished along canonical axis 1, which was related mainly to eugenol, isoeugenol, syringol, 4-methylsyringol, and 4-allylsyringol, as can be deduced from their total canonical structure coefficients (**Figure 3**).

Not much data can be found on the levels of these compounds in green oak wood, probably because some of them are sensitive to seasoning and toasting processes in cooperage, especially syringol and its 4-ethyl and 4-allyl derivatives (7, 36). However, compounds such as guaiacol or 4-methylguaiacol, and especially eugenol, are quite important sensorially in wood-wine interaction (37), and their sensitivity to cooperage operations is at least discussed. The guaiacol and 4-methylguaiacol concentrations showed by *Q. pyrenaica* green wood in this work are similar

Table 3. GC Quantitative Evaluation of Volatile Phenols (Micrograms per Gram of Wood) in Green Oak Wood from *Q. pyrenaica* of Different Regions of Provenance in Spain^a

compound	selected ion <i>m/z</i>	region of provenance			
		Gata/Peña de Francia	Guadarrama	Cantábrica mountain range	Sistema Ibérico
guaiacol	124	0.21 ± 0.08a	0.16 ± 0.22a	0.24 ± 0.31a	0.16 ± 0.14a
4-methylguaiacol	138	0.76 ± 0.58a	0.36 ± 0.41a	0.52 ± 0.56a	0.39 ± 0.26a
4-ethylguaiacol ^b	137	0.07 ± 0.04a	0.02 ± 0.01a	0.14 ± 0.37a	0.02 ± 0.01a
4-vinylguaiacol ^b	135	0.67 ± 0.24a	0.64 ± 0.36a	0.72 ± 0.34a	0.52 ± 0.12a
phenol	94	0.28 ± 0.09a	0.25 ± 0.15a	0.23 ± 0.12a	0.27 ± 0.12a
eugenol		5.71 ± 4.11a	2.27 ± 1.26b	1.47 ± 0.82b	1.57 ± 1.08b
isoeugenol	164	0.71 ± 0.26a	0.28 ± 0.08b	0.26 ± 0.10b	0.32 ± 0.12b
syringol		0.51 ± 0.17a	0.17 ± 0.10b	0.23 ± 0.14b	0.21 ± 0.15b
4-methylsyringol	168	0.69 ± 0.31a	0.27 ± 0.19b	0.24 ± 0.10b	0.35 ± 0.24b
4-allylsyringol	194	3.22 ± 2.66a	1.06 ± 1.74b	0.91 ± 0.92b	0.77 ± 0.65b

^a Average and standard deviation ($x \pm sd$) were calculated for the number of samples available of each region of provenance. Different letters in a row denote a significant difference with 95% confidence level in the Student–Newman–Keuls multiple-range test. ^b Expressed as 4-methylguaiacol equivalent.

Table 4. GC Quantitative Evaluation of Lactones, Furanic Compounds, Pyranones, and Other Volatile Compounds (Micrograms per Gram of Wood) in Green Oak Wood from *Q. pyrenaica* of Different Regions of Provenance in Spain^a

compound	selected ion <i>m/z</i>	region of provenance			
		Gata/Peña de Francia	Guadarrama	Cantábrica mountain range	Sistema Ibérico
2-furanyl-1-ethanone		0.46 ± 0.30a	0.10 ± 0.04b	0.24 ± 0.11b	0.24 ± 0.08b
butyrolactone	86	0.57 ± 0.49ab	0.21 ± 0.08b	0.49 ± 0.27ab	1.06 ± 1.53a
benzaldehyde		0.15 ± 0.08c	0.12 ± 0.06c	0.25 ± 0.08b	0.39 ± 0.10a
hydroxybenzaldehyde ^b	122	0.01 ± 0.005a	0.005 ± 0.004ab	0.002 ± 0.002b	0.002 ± 0.002b
benzothiazole	135	0.10 ± 0.08ab	0.14 ± 0.06a	0.06 ± 0.05b	0.05 ± 0.03b
1 <i>H</i> -pyrrole-2-carboxaldehyde	95	0.02 ± 0.01a	0.03 ± 0.02a	0.02 ± 0.01a	0.03 ± 0.01a
phenylmethanol		1.99 ± 1.36c	1.14 ± 1.69c	5.11 ± 1.51b	6.72 ± 1.10a
2-phenylethanol		0.32 ± 0.17a	0.46 ± 0.15a	0.46 ± 0.27a	0.32 ± 0.11a
2-phenoxyethanol	94	0.13 ± 0.07b	0.11 ± 0.04b	0.19 ± 0.09ab	0.25 ± 0.20a
maltol	126	0.27 ± 0.05a	0.24 ± 0.06a	0.23 ± 0.04a	0.24 ± 0.09a
isomaltol ^c	126	0.14 ± 0.01a	0.13 ± 0.01a	0.12 ± 0.04a	0.11 ± 0.05a
furfural	96	2.42 ± 1.49a	2.35 ± 1.05a	1.35 ± 1.18a	1.91 ± 1.61a
5-methylfurfural		0.25 ± 0.06bc	0.19 ± 0.04c	0.33 ± 0.20ab	0.41 ± 0.26a
furfuryl alcohol	98	0.25 ± 0.13b	0.17 ± 0.02b	0.27 ± 0.19b	0.51 ± 0.47a
5-hydroxymethylfurfural		3.81 ± 2.42a	1.70 ± 0.85a	2.70 ± 3.35a	1.67 ± 1.18a
<i>trans</i> -β-methyl-γ-octalactone	99	6.38 ± 8.01ab	0.84 ± 1.41b	7.32 ± 13.2ab	29.37 ± 66.54a
<i>cis</i> -β-methyl-γ-octalactone	99	59.0 ± 65.7a	15.3 ± 30.02a	26.7 ± 40.6a	14.35 ± 11.77a
<i>cis/trans</i> ratio		11.8 ± 9.98ab	17.4 ± 10.6a	7.48 ± 11.4ab	4.89 ± 5.22b

^a Average and standard deviation ($x \pm sd$) were calculated for the number of samples available of each region of provenance. Different letters in a row denote a significant difference with 95% confidence level in the Student–Newman–Keuls multiple-range test. ^b Expressed as benzaldehyde equivalent. ^c Expressed as maltol equivalent.

Table 5. GC Quantitative Evaluation of Other Lignin-Derived Compounds (Micrograms per Gram of Wood) in Green Oak Wood from *Q. pyrenaica* of Different Regions of Provenance in Spain^a

compound	selected ion <i>m/z</i>	region of provenance			
		Gata/Peña de Francia	Guadarrama	Cantábrica mountain range	Sistema Ibérico
HMPA ^b	137	0.65 ± 0.48a	0.30 ± 0.07b	0.27 ± 0.25b	0.26 ± 0.16b
acetovanillone	151	0.50 ± 0.16a	0.26 ± 0.10b	0.24 ± 0.08b	0.20 ± 0.06b
HMPP ^b	137	1.23 ± 0.98a	0.27 ± 0.13b	0.33 ± 0.16b	0.31 ± 0.12b
propiovanillone	151	4.48 ± 2.50a	2.98 ± 1.33b	1.86 ± 0.64b	1.51 ± 1.04b
HMPB ^b	137	0.09 ± 0.10a	0.04 ± 0.02a	0.07 ± 0.08a	0.03 ± 0.01a
butyrovaniillone ^b	151	0.08 ± 0.04a	0.04 ± 0.01b	0.04 ± 0.02b	0.03 ± 0.01b
methyl vanillyl ether ^c	137	0.83 ± 0.43a	0.47 ± 0.18b	0.41 ± 0.17b	0.30 ± 0.15b
HDMPA ^d	167	0.47 ± 0.32a	0.34 ± 0.11a	0.32 ± 0.22a	0.36 ± 0.08a
ethyl vanillyl ether ^c	137	2.14 ± 0.87a	1.35 ± 0.62b	1.37 ± 0.47b	0.93 ± 0.23b
acetosyringone	181	0.53 ± 0.15a	0.30 ± 0.11b	0.37 ± 0.17b	0.33 ± 0.14b
HDMPP ^d	167	1.60 ± 0.79a	0.47 ± 0.25b	0.74 ± 0.38b	0.76 ± 0.42b
propiosyringone ^d	181	1.60 ± 1.78a	0.66 ± 0.31b	0.60 ± 0.35b	0.42 ± 0.28b

^a Average and standard deviation ($x \pm sd$) were calculated for the number of samples available of each region of provenance. Different letters in a row denote a significant difference with 95% confidence level in the Student–Newman–Keuls multiple-range test. HMPA, 2-(4-hydroxy-3-methoxyphenyl) acetaldehyde; HMPP, 1-(4-hydroxy-3-methoxyphenyl)-2-propanone; HMPB, 1-(4-hydroxy-3-methoxyphenyl)-2-butanone; HDMPA, 2-(4-hydroxy-3,5-dimethoxyphenyl) acetaldehyde; HDMPP, 1-(4-hydroxy-3,5-dimethoxyphenyl)-2-propanone. ^b Expressed as acetovanillone equivalent. ^c Expressed as vanillin equivalent. ^d Expressed as acetosyringone equivalent.

Table 6. GC Quantitative Evaluation of Volatile Compounds Related to Off-flavors (Micrograms per Gram of Wood) in Green Oak Wood from *Q. pyrenaica* of Different Regions of Provenance in Spain^a

compound	selected ion <i>m/z</i>	region of provenance			
		Gata/Peña de Francia	Guadarrama	Cantábrica mountain range	Sistema Ibérico
1-hexanal	56	1.12 ± 0.56a	1.85 ± 0.98a	1.21 ± 1.14a	1.10 ± 0.47a
1-hexanol	56	0.55 ± 1.07a	0.10 ± 0.04a	0.28 ± 0.14a	0.44 ± 0.27a
1-nonanal	57	0.21 ± 0.06ab	0.14 ± 0.04b	0.25 ± 0.15a	0.28 ± 0.07a
(<i>E</i>)-2-octenal	70	0.54 ± 0.19a	0.44 ± 0.16ab	0.36 ± 0.13b	0.39 ± 0.14ab
2-ethyl-1-hexanol	57	0.83 ± 0.30b	0.72 ± 0.15b	1.22 ± 0.25a	1.05 ± 0.79ab
(<i>E</i>)-2-nonenal	70	14.4 ± 4.05a	10.5 ± 6.31b	8.25 ± 2.72b	8.50 ± 2.71b

^a Average and standard deviation ($x \pm sd$) were calculated for the number of samples available of each region of provenance. Different letters in a row denote a significant difference with 95% confidence level in the Student–Newman–Keuls multiple-range test.

to those found by us in green and seasoned oak woods from different species and provenances (7), in which no significant variations or only small concentration increases during natural seasoning have been described. During toasting, these compounds are formed at relatively high temperatures, mainly on the wood surface (36), so they could not be formed when light

toasting is applied, especially guaiacol (7). Therefore, the levels of these compounds in green woods could be important in a first approach to ecological characterization of this wood.

Most of the studies reporting on volatile phenols in green oak wood are concerned only with eugenol. This compound showed highly variable levels in green oak wood, depending

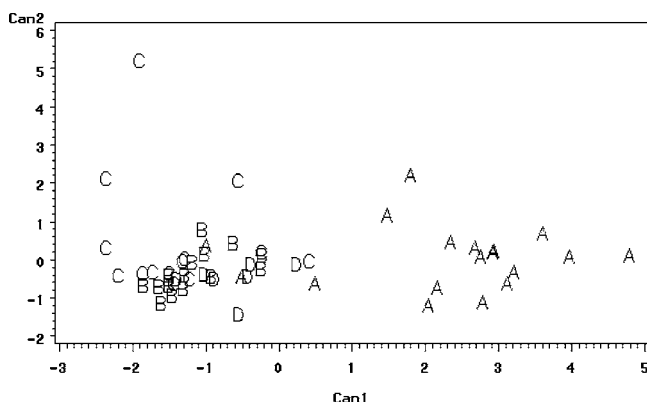


Figure 3. Canonical discriminant analysis of volatile phenols in green wood of *Q. pyrenaica*, from different regions of provenance in Spain: A, Gata/Peña de Francia; B, Guadarrama; C, Cantábrica mountain range; D, Sistema Ibérico (98.29% of dispersion; canonical correlations of 0.86 and 0.36 and eigenvalues of 2.94 and 0.15 for Can 1 and Can 2, respectively). The total canonical structure coefficients of functions 1 and 2 were, respectively, as follows: guaiacol, 0.04 and 0.44; 4-methylguaiacol, 0.34 and 0.40; 4-ethylguaiacol, -0.04 and 0.71; 4-vinylguaiacol, 0.01 and 0.50; phenol, 0.21 and -0.16; eugenol, 0.68 and 0.01; isoeugenol, 0.90 and 0.08; syringol, 0.83 and 0.36; 4-methylsyringol, 0.77 and -0.04; 4-allylsyringol, 0.58 and 0.14.

on the species, origin, and tree, with average values ranging from 0 to 10 $\mu\text{g/g}$, in general higher in *Q. petraea* than in *Q. robur* (7, 21, 30). The levels in *Q. pyrenaica* from Guadarrama, the Cantábrica mountain range, and Sistema Ibérico regions were similar to those described in *Q. robur*, whereas levels from the Gata/Peña de Francia region were similar to those of *Q. petraea*, from both French and Spanish forests. The cooperage process's influence on this compound is also discussed, but it is accepted that seasoning and toasting in general produce only small variations in its concentrations, decreasing or increasing during seasoning (16, 38–40) or toasting (7, 41), depending on wood characteristics and cooperage conditions. Therefore, knowledge of its levels in green wood can be important in predicting the wood aromatic potential in wood–wine interaction.

β -Methyl- γ -octalactones, Furanic Compounds, Cyclic Ketones, Pyrrole Structures, and Other Related Compounds. **Table 4** shows the concentrations of some compounds, with different chemical structures, found in the wood, most of them highly sensitive to the seasoning and toasting process in cooperage (7, 18). With the exception of lactones, they are not very interesting from the enological point of view due to the low concentrations showed in green woods, but they could be important because they may give an idea of the natural variability of the wood, especially if we bear in mind their contribution to the organoleptic complexity of wine. Most of them show low concentrations in *Q. pyrenaica*, except phenylmethanol, some furanic derivatives, and the *cis* and *trans* isomers of β -methyl- γ -octalactone, whereas only phenylmethanol and *trans*- β -methyl- γ -octalactone showed significant differences among provenance regions (**Table 4**). Taking into account the canonical discriminant analysis carried out with these compounds, four groups corresponding to different studied provenance regions can be distinguished in the canonical graphical representation (**Figure 4**). This happens along canonical axis 1, which was related mainly to levels of phenylmethanol, benzaldehyde, and 5-methylfurfural, according to the total canonical structure coefficients of discriminant function 1.

Looking more closely at the levels of *cis*- and *trans*- β -methyl- γ -octalactones, we found a high dispersion of data, with high

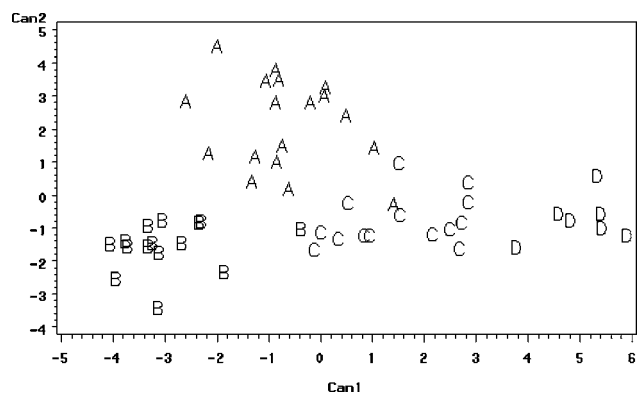


Figure 4. Canonical discriminant analysis of lactones, furanic compounds, pyranones, and other volatile compounds in green wood of *Q. pyrenaica*, from different regions of provenance in Spain: A, Gata/Peña de Francia; B, Guadarrama; C, Cantábrica mountain range; D, Sistema Ibérico (93.14% of dispersion; canonical correlations of 0.93 and 0.85 and eigenvalues of 6.97 and 2.68 for Can 1 and Can 2, respectively). The total canonical structure coefficients of functions 1 and 2 were, respectively, as follows: 2-furanyl-1-ethanone, 0.13 and 0.69; butyrolactone, 0.38 and 0.14; benzaldehyde, 0.78 and -0.18; hydroxybenzaldehyde, -0.38 and 0.53; benzothiazole, -0.47 and -0.07; 1H-pyrrole-2-carboxaldehyde, 0.03 and -0.07; phenylmethanol, 0.84 and -0.21; 2-phenylethanol, -0.13 and -0.31; 2-phenoxyethanol, 0.47 and -0.11; maltol, -0.09 and 0.30; isomaltol, -0.32 and 0.15; furfural, -0.22 and 0.16; 5-methylfurfural, 0.52 and -0.03; furfuryl alcohol, 0.46 and 0.02; HMF, -0.02 and 0.41; *trans*- β -methyl- γ -octalactone, 0.33 and 0.001; *cis*- β -methyl- γ -octalactone, -0.05 and 0.45; *cis/trans* ratio -0.43 and -0.06.

standard deviations related to average values. In general, the concentrations of the *cis* isomer were higher than those of the *trans* isomer, except in some trees from the Sistema Ibérico region. The interindividual variability was huge, with values between 0.006 and 179 $\mu\text{g/g}$ for the *trans* isomer and values between 0.033 and 251 $\mu\text{g/g}$ for the *cis* isomer, even when the trees were grown under similar conditions in the same forest. Similar results were obtained by Masson et al. (42) in French oak trees from the Limousin, Tronçais, Jupilles, and Darney forests, in *Q. robur* as much as in *Q. petraea*, and in American oak trees from Virginia and Missouri forests, with coefficients of variation between 36 and 94%. Doussot et al. (16) found higher coefficients of variation, 162% for *Q. robur* and 96% for *Q. petraea*, from six different French forests, with 133 trees studied. This variability among individuals means that no significant statistical differences were found among regions. Although its concentrations are sensitive to cooperage operations such as seasoning and toasting (7, 32, 36, 38), some authors described an important oak effect, because the concentrations of these compounds in wines reflect those observed for the oak prior to coopering, so coopering variability does not appear to have any significant effect on oak lactone concentration in the wines (16, 43). Therefore, like eugenol, its levels in green wood can be decisive in aromatic potential of the wood in wood–wine interaction.

Other Lignin-Derived Compounds. The phenyl ketones and their respective acetaldehyde and 2-one isomers (**Table 5**), together with phenolic aldehydes previously studied, are a compound group related to the metabolism of lignin, sensitive to the seasoning and toasting process in cooperage (7). Although the phenolic aldehydes play a major role in oak wood aromatic potential, and they are the only ones considered in other studies on the enological characterization of oak wood, the contribution of all these other compounds to the aromatic potential of wood cannot be considered to be negligible, because they include

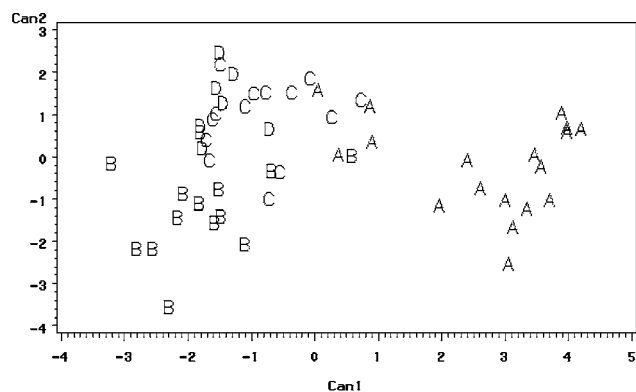


Figure 5. Canonical discriminant analysis of other lignin-derived compounds in green wood of *Q. pyrenaica*, from different regions of provenance in Spain: A, Gata/Peña de Francia; B, Guadarrama; C, Cantábrica mountain range; D, Sistema Ibérico (95.07% of dispersion; canonical correlation of 0.89 and 0.69 and eigenvalues of 4.05 and 0.93 for Can 1 and Can 2, respectively). The total canonical structure coefficients of functions 1 and 2 were, respectively, as follows: HMPA, 0.54 and -0.16 ; acetovanillone, 0.79 and -0.28 ; HMPP, 0.69 and -0.09 ; propiovanillone, 0.53 and -0.47 ; HMPB, 0.31 and 0.06; butyrovannillone, 0.70 and -0.12 ; methyl vanillyl ether, 0.62 and -0.31 ; HDMPA, 0.31 and -0.05 ; ethyl vanillyl ether, 0.59 and -0.23 ; acetosyringone, 0.65 and 0.07; HDMP, 0.74 and 0.09; propiosyringone, 0.61 and -0.21 .

compounds with similar chemical structures that can add their aromatic properties, and even display synergy among them or with other aromatic compounds, because they also are extracted during wood–wine interaction. The levels of the majority of them were significantly higher in *Q. pyrenaica* from Gata/Peña de Francia than in the other regions, according to the Newman–Keuls test in **Table 5**. Again, as in the case of volatile phenols, this region is easily differentiated by means of canonical discriminant analysis, shown in **Figure 5**. We can discriminate two groups of samples: the Gata/Peña de Francia region and all others, along canonical axis 1, which was related to the majority of the studied compounds, as can be deduced from their canonical structure coefficients.

Volatile Compounds Related to Off-flavors. Finally, **Table 6** shows the average concentrations of some compounds related to off-flavors in wines stored in new barrels: 1-hexanal (herbaceous, sawdust), 1-hexanol (vegetable), 1-nonanal (rancid, metallic), (*E*)-2-octenal (green walnut, sawdust), 2-ethyl-1-hexanol (earthy, vegetable, solvent), and (*E*)-2-nonenal (green walnut, sawdust) (44). The most abundant was (*E*)-2-nonenal, with average concentrations between 8.25 and 14.4 $\mu\text{g/g}$ of wood, with significant differences only in the case of the Gata/Peña de Francia region in relation to the other ones. As far as we know, the presence of these compounds in green oak wood from different species and origins has never been studied and, therefore, we do not know if concentrations found by us are high or low with regard to other oak woods. Neither have we found data about their evolution during natural seasoning. Only with regard to toasting do we know that their concentrations decrease as the intensity of toasting is increased (44).

In view of all our results, the tannic, phenolic, and volatile composition of green *rebollo* oak wood from Spain was similar to those of other *Quercus* species commonly used in cooperage to make barrels, showing only small quantitative differences more significant with respect to American than to French species. The four provenance regions studied showed similar chemical composition, with high variability among individuals,

often higher than the variability among regions of provenance, but in line with that described in other European and American oak woods. The highest differences among regions were shown in minor enological compounds, either for their small aromatic potential or their sensitivity to coopering (seasoning and toasting). Therefore, *Q. pyrenaica* wood should be considered to be suitable to make barrels for aging wine. Besides which, obtaining chips from oak trees with small diameters or defects that are unsuitable for stave-making remains feasible, which could be advantageous as a short-term yield in *Q. pyrenaica* oak wood forests.

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