Changes in Low Molecular Weight Phenolic Compounds in Spanish, French, and American Oak Woods during Natural Seasoning and Toasting

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The evolution of low molecular weight polyphenols in Spanish oak heartwood of *Quercus robur*, *Quercus petraea*, *Quercus pyrenaica*, and *Quercus faginea* was studied by HPLC, in relation to the processing of wood in barrel cooperage. The polyphenolic composition of Spanish woods subjected to natural seasoning for 3 years and to the toasting process was studied in relation to those of French oak of *Q. robur* (Limousin) and *Q. petraea* (Allier) and American oak of *Q. alba* (Missouri), which are habitually used in cooperage. The concentrations of benzoic and cinnamic acids and aldehydes of Spanish woods increased during seasoning depending on the duration of this process and in the same way as those of French and American woods. The process having the main influence on the phenolic composition of wood was the toasting. It led to high increases in the concentration of phenolic aldehydes and acids, especially cinnamic aldehydes (sinapic and coniferylic aldehydes), followed by benzoic aldehydes (syringaldehyde and vanillin) and benzoic acids (syringic and vanillic acids). This polyphenolic composition in Spanish oak species evolved during toasting as in French and American oak, but quantitative differences were found, which were especially important in American species with respect to the others.

INTRODUCTION

The extraction of different polyphenolic and volatile compounds from oak barrels into wine during its aging depends mainly on the pool of potential extractable compounds originally present in the barrel wood. The chemical composition of this oak wood is influenced by two groups of main factors, on the one hand, the oak species, the geographical origin, and the sylvocultural treatment of the tree (*1−10*), and, on the other, the processing of the wood in cooperage, that is, the method used to obtain the staves, the method of seasoning (natural or artificial, length, and location) (*1, 11−15*), and the degree of oak toasting during the barrel's manufacturing (*16−23*).

Seasoning results in the dehydration of wood, until its humidity rate is in balance with the ambient humidity. However, in cooperage, the natural seasoning is much more than a stage in the dehydration of wood, necessary to keep the pieces that together make up a barrel. It is, basically, a refining stage, comparable to the slow and complex ripening of grapes (*24*). Unseasoned and aggressive wood becomes dry and aromatic wood. Seasoning produces a loss of hydrosoluble polyphenolic substances, such as the ellagitannins. This effect could be due to different mechanisms: a rain leaching of the staves (*25*), a hydrolytic oxidative degradation process (*13, 14*) with the formation of brown polymers (26), an insolubilization of oligomeric ellagitannins after their polymerization (*26, 27*), and also an enzymatic activity (phenol heterosidase, etherase, and depsidase) of fungic nature (*28, 29*) with the destruction of heterosydic phenolic structures, such as coumarins and hydrolyzable tannins (*30*).

Seasoning can have also a significant effect on the aromatic profile of wood. In comparison with the wood heat treatment, its contribution to the pool of aromatic compounds, which can be liberated into the wine, appears to be limited but not negligible. The influence of seasoning is more evident in the variation of the oak lactone concentrations, but changes in the levels of volatile phenols, such as eugenol, and phenolic aldehydes (vanilline, syringaldehyde, coniferaldehyde, and sinapaldehyde) are also observed during seasoning (*11, 14, 31*). Benzoic aldehydes can be oxidized to the correspondent phenolic acids (vanillic and syringic acids) (*14, 32*).

In addition to the wood seasoning, the methods used in cooperage affect substantially the physicochemical properties of the wood. The process of barrel toasting probably has the main influence on the chemical compounds of the wood, which are susceptible to migrate into wine or spirits during their aging, affecting their organoleptic properties. The heat treatment of oak staves is a technique necessary to facilitate stave assembly. Although lignin is very thermomalleable, heating alone is not enough to affect glycosidic polymers (cellulose and hemicelluloses); therefore, a combination of heating and humidity must be applied during stave assembly. A second heating (toasting) is carried out to help a superficial degradation of oak wood that produces new aromatic compounds (*16, 17, 33−36*). Both the quality and the quantity of the oak wood extractable substances vary with the toasting level; therefore,

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mastery and repeatability are vital in the production of quality barrels (*18*, *19*).

During these processes, a variety of hydrothermolysis and pyrolysis reactions take place, producing the degradation of biopolymers, such as lignin, polyosides, polyphenols, and lipids. The thermodegradation of lignin leads to the formation of mainly volatile phenols and phenolic aldehydes and some phenolic alcohols. Whereas the nontoasted oak wood has only a small quantity of volatile phenols, mainly eugenol, and traces of phenolic aldehydes, in the toasted wood a variety of mono- and dimethoxylated phenols and high levels of benzoic and cinnamic aldehydes arise (*17*, *18*, *20*, *21*, *32*, *33*–*37*). The aldehydes come from thermic degradation of the terminal monomeric units of lignin: the cinnamic aldehydes become benzoic aldehydes, and these are oxidized to phenolic acids. The last one by decarboxylation can become volatile phenols. Toasting can also produce phenyl ketones and some esters of phenolic acids, but their organoleptic role is not important (*36*).

On the other hand, heat treatment of oak wood also results in a decrease in the amount of ellagitannins (castalin and castalagin) and a simultaneous increase in ellagic acid (*20*).

Referring to the polyosides, wood toasting results in sugar degradation, with the formation of furanic aldehydes, compounds with a burnt almond odor (*36*, *38*), mainly furfural and 5-(hydroxymethyl)furfural (*18*, *39*). Other volatile odorous compounds that derive from sugar degradation, a few pentacyclic and hexacyclic ketones, were identified (*18*, *22*, *23*).

The heat degradation of wood lipids leads to lactones, which are regarded as the most important of the oak volatile compounds that contribute to the flavor of barrel-aged alcoholic beverages (*40*). Oak wood presents two isomers, cis and trans, of β-methyl-γ-octalactone, molecules responsible for the characteristic odor of oak, which are present in nontoasted wood. At the beginning of toasting the quantity of *cis*-γ-lactone increases, but if the toasting is lengthy, there can be a total destruction of these compounds (*17*, *18*, *36*, *41*).

The present study is a part of extensive research in which we are studying the chemical characteristics of Spanish oak for use in cooperage (*5*, *6*, *8*, *15*, *42*, *43*). We have studied the polyphenolic composition of wood of four different species grown in Spain, *Quercus robur*, *Quercus petraea*, *Quercus pyrenaica*, and *Quercus faginea*, and we have compared these woods with others of the same species (*Q. robur* and *Q. petraea*) but of a different origin (Limousin and Allier) and with samples of different species and origin (American *Q. alba*). We have also studied the phenolic compound evolution of the Spanish oak wood during the first year of natural seasoning. In this work, we present the results obtained on the evolution of low molecular weight polyphenolic composition of Spanish oak wood, after 3 years of natural seasoning and after toasting, compared with French and American oak woods used in barrel production.

MATERIALS AND METHODS

Collection of Wood Samples. Heartwood of *Q. robur* (seven trees), *Q. petraea* (three trees), *Q. pyrenaica* (five trees), and *Q. faginea* (three trees) grown in the Alava province of Spain were provided by Centro Técnico de la Madera del PaísVasco, S.A. This wood was sent to Demptos Cooperage (Bordeaux, France) (average annual temperature = 12.5 °C;

total precipitation = 950 mm/year; average over 50 years), where the manufacturer proceeded to obtain the staves, carried out the process of natural seasoning in the open air during 3 years, and made the barrels (about four barrels of each species). During the process, the staves were toasted at medium intensity level (35 min, temperature on the wood surface 160–170 °C), in the traditional way over a wood fire, according to the process used at the cooperage (*44*). Heartwood staves of French oak of *Q. robur* (Limousin) and *Q. petraea* (Allier), before and after toasting, and of American oak of *Q. alba* (Missouri), after toasting, were also provided by Demptos Cooperage.

Wood samples were taken from the nontoasted and toasted staves, four pieces of each species and each geographical origin, except for *Q. alba* from America, from which we only took two. Three pieces of wood were cut from the center and the headboards of the staves. In the pieces obtained from toasted staves, the layer of toasted wood was cut off to a depth of 4 mm with an electric rotary saw. The wood pieces were ground and sieved, taking the sawdust ranging in size from 0.80 to 0.28 mm.

Extraction. The sawdust samples (1 g) were extracted with 100 mL of methanol/water (1:1) at room temperature for 24 h, according to the method described by Fernández de Simón et al. (*5*).

Standards. Reference compounds were purchased from Fluka (gallic acid, aesculetin, and scopoletin), Aldrich (vanillic, ferulic, and 2-furanoic acids, syringaldehyde, sinapaldehyde, and coniferaldehyde), Apin (ellagic acid), Chem Service (syringic acid), Merck (vanillin), and Sigma [5-(hydroxymethyl)-furfural].

HPLC Analysis. HPLC analysis of diethyl ether and ethyl acetate extracts was carried out according to the method of Conde et al. (*45*). An apparatus equipped with a diode array detector and with a C_{18} Hypersyl ODS (5 μm) column (20 cm × 4 mm i.d.), protected with a precolumn of the same material, was used. The elution conditions were modified as follows: flow rate, 1 mL/min; temperature, 30 °C; solvent A = H_2O/PO_4H_3 (999:1), solvent B = $MeOH/PO_4H_3$ (999:1); linear gradient from 0 to 100% B, in 70 min; detection at 325 nm (with a bandwidth of 150 nm). UV spectra (240–400 nm) were also recorded. Chromatographic peaks were identified by comparing their retention time and the UV spectra with those of reference compounds. Quantitative determinations were carried out from diethyl ether and ethyl acetate extracts by the external standard method.

Statistical Analysis. Univariate analysis was carried out, using the BMDP-7D (ANOVA) program (WJ Dixon, BMDP Statistical Software, software release, 1990). Average and standard deviation were calculated for each variable of each species and origin, using a single-variable model. The Student–Newman–Keuls multiple-range test was also carried out to determine the significance levels of the differences of the variables considering groups by wood stages (nontoasted and toasted wood), species, and origins. Canonical discriminant analysis was also carried out with all of the phenols evaluated, using the CAND.SAS program (SAS Institute Inc., SAS/STAT, version 6, 4th ed., 1994).

RESULTS

Oak Wood after 3 Years of Natural Seasoning. The HPLC analysis of the diethyl ether and ethyl acetate extracts revealed the presence of the following components: gallic, vanillic, syringic, ferulic, and ellagic acids; vanillic, syringic, coniferylic, and sinapic aldehydes; and the coumarins aesculetin and scopoletin, in all samples of seasoned woods. Moreover, other low molecular weight phenols, such as those derived from ellagic acid and from gallic acid, reported by Fernández de Simón et al. (*5*), were found. Also, the furanic compounds 2-furanoic acid and 5-(hydroxymethyl)furfural were identified. Figure 1a shows an example of

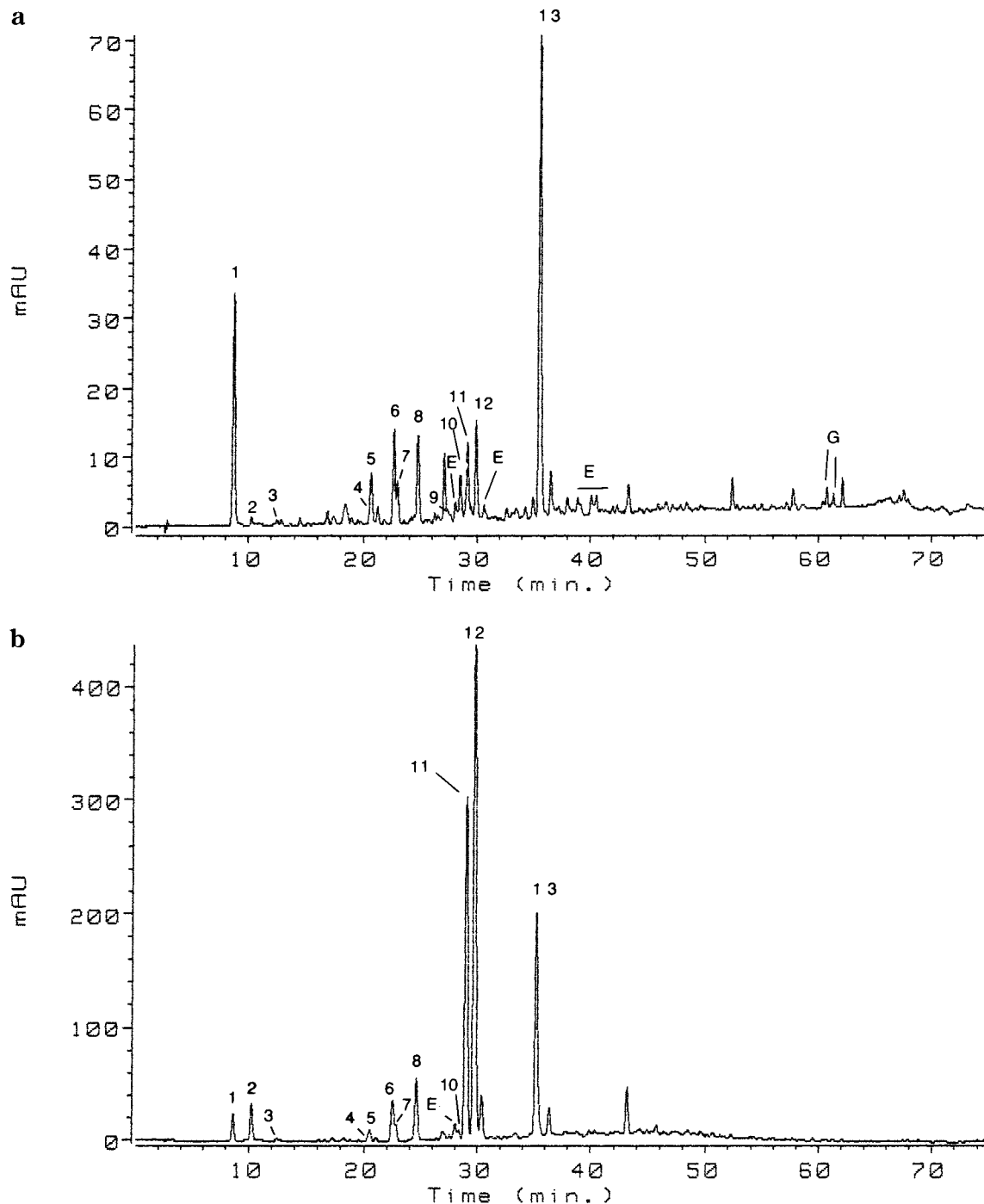**1792** *J. Agric. Food Chem.,* Vol. 49, No. 4, 2001

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Figure 1. HPLC chromatograms (325 ± 75 nm) of ether extracts from seasoned (a) and toasted (b) heartwoods of *Quercus* sp.: (1) gallic acid; (2) 5-(hydroxymethyl)furfural; (3) 2-furanoic acid; (4) aesculetin; (5) vanillic acid; (6) vanillic aldehyde; (7) syringic acid; (8) syringaldehyde; (9) scopoletin; (10) ferulic acid; (11) coniferaldehyde; (12) sinapaldehyde; (13) ellagic acid; (E) derived from ellagic acid; (G) derived from gallic acid.

HPLC chromatograms of ether extracts from Spanish seasoned woods.

The HPLC quantitative evaluations of the phenolic acids and aldehydes and of the coumarins for the samples from Spanish, French, and American seasoned woods are included in Table 1. The evaluations of aesculetin and vanillic acid were carried out using specific wavelengths of 340 and 261 nm, respectively, because their retention times were similar in our HPLC analysis conditions. The peaks of ferulic acid, in the American oak wood chromatograms, and of scopoletin, in all chromatograms, were contaminated with one ellagitannin, and their quantification was not possible,

because, in this case, there was no specific wavelength for each compound.

As can be seen in Table 1, the main wood component in all species was ellagic acid and the second one was gallic acid, whereas ferulic acid, sinapic aldehyde, and coumarins were present in the lowest concentrations. We must emphasize the high standard deviations of most of the variables in all woods analyzed (Table 1), which can be responsible for the few or unremarkable significant differences among species and origins, according to the variance analysis (Table 3). We applied a Newman–Keuls multiple-range test to determine which means are significantly different from others. The

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Table 1. Low Molecular Weight Phenolic Compounds, Expressed as Micrograms per Gram of Wood, of Spanish, French, and American Oak Woods after 3 Years of Natural Seasoning[a]

| | Spanish oak | | | | French oak | | American oak |
	Q. robur	*Q. petraea*	*Q. pyrenaica*	*Q. faginea*	*Q. robur*	*Q. petraea*	*Q. alba*
acids							
gallic	181 ± 168	285 ± 168	445 ± 149	582 ± 438	243 ± 144	72 ± 51	103 ± 70
vanillic	12.0 ± 2.7	13.9 ± 3.3	14.2 ± 3.1	9.2 ± 1.7	14.8 ± 2.2	11.7 ± 1.6	13.4 ± 3.2
syringic	8.6 ± 3.4	9.6 ± 3.5	15.0 ± 2.4	11.3 ± 2.6	17.9 ± 2.8	12.5 ± 2.4	24.4 ± 11.6
ferulic	2.7 ± 0.5	2.8 ± 1.7	2.0 ± 0.4	2.4 ± 1.3	4.1 ± 1.4	2.8 ± 1.0	nd[b]
ellagic	736 ± 218	547 ± 284	735 ± 186	790 ± 99	592 ± 163	628 ± 144	746 ± 60
aldehydes							
vanillin	10.7 ± 0.7	13.5 ± 2.2	15.0 ± 1.0	6.8 ± 1.6	5.6 ± 1.8	8.6 ± 2.2	13.4 ± 2.9
syringic	16.7 ± 6.1	20.1 ± 4.4	21.0 ± 6.5	13.1 ± 3.5	8.1 ± 1.4	12.8 ± 2.9	20.7 ± 2.3
coniferylic	10.1 ± 2.6	11.7 ± 3.4	7.5 ± 1.2	4.6 ± 1.2	6.2 ± 1.9	6.4 ± 3.8	5.9 ± 0.5
sinapic	2.7 ± 0.6	3.4 ± 0.5	2.9 ± 0.9	1.9 ± 0.7	1.9 ± 0.7	2.2 ± 0.7	4.2 ± 1.1
coumarins							
aesculetin	1.2 ± 1.0	3.7 ± 1.2	1.9 ± 0.9	1.4 ± 0.8	2.8 ± 2.9	1.2 ± 0.7	5.0 ± 3.5
other compounds							
5-OMe-furfural	$5.1. \pm 2.3$	8.2 ± 5.7	4.8 ± 2.1	8.4 ± 5.6	$0.9. \pm 1.9$	1.8 ± 1.6	2.8 ± 2.1
2-furanoic acid	$1.8. \pm 0.8$	2.4 ± 0.9	0.3 ± 0.6	2.9 ± 0.7	$0.7. \pm 0.7$	1.6 ± 1.1	16.1 ± 3.0

[a] Average and standard deviation ($x \pm$ SD) were calculated for four samples in Spanish and French oak woods and for two samples in American oak wood. [b] nd, not determined.

Table 2. Low Molecular Weight Phenolic Compounds, Expressed as Micrograms per Gram of Wood, of Spanish, French, and American Oak Woods after Toasting[a]

| | Spanish oak | | | | French oak | | American oak |
	Q. robur	*Q. petraea*	*Q. pyrenaica*	*Q. faginea*	*Q. robur*	*Q. petraea*	*Q. alba*
acids							
gallic	234 ± 151	450 ± 367	361 ± 251	436 ± 134	490 ± 251	196 ± 56	96 ± 7
vanillic	22.8 ± 1.7	22.4 ± 2.1	24.5 ± 2.7	29.9 ± 8.8	21.4 ± 2.9	30.1 ± 5.4	35.5 ± 34.4
syringic	31.7 ± 6.8	29.5 ± 1.6	44.4 ± 6.9	37.0 ± 7.2	43.5 ± 5.4	48.5 ± 10.5	168 ± 19.4
ferulic	nd[b]	nd	nd	nd	nd	nd	nd
ellagic	895 ± 107	916 ± 92	835 ± 95	955 ± 186	1176 ± 215	729 ± 73	753 ± 102
aldehydes							
vanillin	67.2 ± 8.8	56.8 ± 5.6	72.3 ± 13.8	64.6 ± 13.8	57.9 ± 9.2	91.8 ± 16.2	151 ± 14
syringic	138 ± 42	136 ± 27	186 ± 22	119 ± 32	154 ± 13	189 ± 48	536 ± 69
coniferylic	424 ± 20	392 ± 30	386 ± 88	312 ± 86	327 ± 36	545 ± 87	675 ± 81
sinapic	252 ± 49	281 ± 37	265 ± 71	174 ± 20	212 ± 99	342 ± 44	595 ± 27
coumarins							
aesculetin	2.1 ± 1.1	4.2 ± 0.9	1.6 ± 1.3	4.9 ± 3.2	1.68 ± 0.7	1.97 ± 0.4	1.45 ± 0.2
other compounds							
5-OMe-furfural	88 ± 33	117 ± 16	99 ± 66	114 ± 29	201 ± 53	115 ± 20	234 ± 43
2-furanoic acid	5.2 ± 1.8	3.7 ± 1.4	6.5 ± 4.2	4.6 ± 1.4	10.1 ± 0.5	5.0 ± 1.4	23.3 ± 5.9

[a] Average and standard deviation ($x \pm$ SD) were calculated for four samples in Spanish and French oak woods and for two samples in American oak wood. [b] nd, not determined.

same letters in Table 3 indicate nonsignificant differences between each pair of means, and the different letters indicate significant differences at the 95.0% confidence level. Considering each phenolic compound independently, it can be pointed out that seasoned Spanish woods did not present significant differences with respect to French (*Q. robur* or *Q. petraea*) and American seasoned woods (*Q. alba*).

In relation to the variation of the polyphenol contents throughout the seasoning process, if we compare the results obtained from Spanish wood species seasoned during 3 years (Table 1) with those obtained from the same woods but before seasoning and after only 1 year of seasoning (*15*), there was an important increase in the average contents of all compounds, depending on the seasoning time increment. The exceptions were sinapic aldehyde and aesculetin. The mean contents in Spanish oak (average of *Q. robur*, *Q. petraea*, *Q. pyrenaica*, and *Q. faginea*) before seasoning, after 1 year of seasoning (*15*), and after 3 years of seasoning (Table 1) were, respectively, the following: 121, 330, and 373 μg/g gallic acid; 1.86, 4.27, and 12.33 μg/g vanillic acid; 3.06, 8.64, and 11.12 μg/g syringic acid; 0.87, 0.79, and 2.49 μg/g ferulic acid; 194, 279, and 702 μg/g ellagic acid; 2.28, 5.60, and 11.50 μg/g vanillin; 2.78, 8.61, and

17.74 μg/g syringic aldehyde; 3.78, 4.83, and 8.49 μg/g coniferylic aldehyde. The compounds that most increased during seasoning were syringic aldehyde and vanillic acid. In the case of sinapic aldehyde and aesculetin, the concentrations of which increased during the first year of seasoning (*15*), no drastic changes were observed during the second stage of seasoning, although they had a decreasing tendency.

To compare the Spanish woods with French and American woods, at the end of seasoning, we carried out a canonical discriminant analysis considering the phenolic compounds evaluated in Table 1. The resulting mathematical model explained 100% of the total dispersion, which was distributed among six canonical functions. Figure 2 shows the graphical representation of the projections of the points of each group on the plane defined by the two principal canonical axes (functions 1 and 2), which represent a cumulative proportion of 81.5% of the total dispersion, with a canonical correlation of 0.967 and 0.920, and eigenvalues of 14.59 and 5.53, respectively. Discriminant function 1 was related mainly to the contribution of vanillin, syringic aldehyde, syringic acid, coniferylic aldehyde, vanillic acid, and gallic acid, in this sequence, whereas function 2 was related to syringic acid, coniferylic aldehyde, vanillic

Table 3. Student–Newman–Keuls Multiple-Range Test of All the Components of Seasoned Wood and Toasted Wood[a]

	acids				aldehydes				coumarin
	gallic	vanillic	syringic	ellagic	vanillin	syringic	coniferylic	sinapic	aesculetin
nontoasted									
Spanish									
Q. robur	a	a	a	a	a	a	a	a	a
Q. petraea	a	ab	a	a	a	a	a	a	a
Q. pyrenaica	a	ab	a	a	a	a	a	a	a
Q. faginea	a	a	a	ab	a	a	a	a	a
French									
Q. robur	a	ab	a	a	a	a	a	a	a
Q. petraea	a	a	a	a	a	a	a	a	a
American									
Q. alba	a	ab	ab	ab	a	a	a	a	a
toasted									
Spanish									
Q. robur	a	ab	b	ab	b	b	c	c	a
Q. petraea	a	ab	b	ab	b	b	bc	c	a
Q. pyrenaica	a	ab	c	ab	b	c	bc	c	a
Q. faginea	a	b	bc	ab	b	b	b	b	a
French									
Q. robur	a	ab	c	b	b	bc	b	bc	a
Q. petraea	a	b	c	a	c	c	d	d	a
American									
Q. alba	a	b	d	ab	d	d	e	e	a

[a] The different letters denote a statistically significant difference with 95% confidence level.
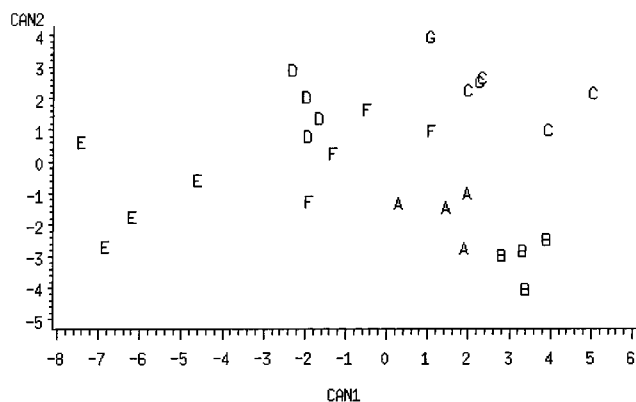

Figure 2. Canonical discriminant analysis of low molecular weight polyphenols of seasoned oak woods; projections of the points of each species and provenance, on the two principal canonical axes: (A) *Q. robur*, (B) *Q. petraea*, (C) *Q. pyrenaica*, and (D) *Q. faginea*, from Spain; (E) *Q. robur* and (F) *Q. petraea*, from France; (G) *Q. alba* from America. Eigenvalues for discriminant functions 1 and 2 were 14.59 and 5.53, respectively. Standardized coefficients of discriminant functions 1 and 2 were as follows for each variable: gallic acid, −0.6 and 0.04; vanillic acid, −0.7 and −1.2; syringic acid, −1.4 and 1.7; ellagic acid, −0.3 and 0.1; vanillin, 2.4 and 1.0; syringaldehyde, 1.6 and 0.9; coniferaldehyde, −0.8 and −1.4; sinapaldehyde, 0.3 and −0.6; aesculetin, −0.3 and 0.1, respectively.

acid, vanillin, and syringic aldehyde, in this sequence. The standardized coefficients of each variable for discriminant functions 1 and 2 are included in the Figure 2 legend. The sets of points showed a pattern of distribution in which seven well-defined groups can be distinguished, related to the species and origin of woods; especially, function 1 distinguished *Q. robur* with respect to all other woods. Only the set of points of Spanish *Q. pyrenaica* and American *Q. alba* overlapped.

In relation to ellagic and gallic acid derivatives, which appeared in green woods (*15*), they were hardly found in the samples, after 3 years of seasoning (Figure 1a).

Oak Wood after Toasting. The HPLC chromatograms of diethyl ether and ethyl acetate extracts from toasted woods revealed the presence of the same components that were identified in seasoned woods before

toasting: gallic, vanillic, syringic, ferulic, and ellagic acids; vanillic, syringic, coniferylic, and sinapic aldehydes; and the coumarins aesculetin and scopoletin. The furanic compounds, 2-furanoic acid and 5-(hydroxymethyl)furfural, and a few derivatives of ellagic acid and gallic acid were also found. Figure 1b shows an example of the HPLC chromatograms of diethyl ether extracts from toasted Spanish woods. It can be observed that the chromatographical patterns related to toasted woods and nontoasted woods were very different.

The HPLC quantitative evaluations of phenolic acids and aldehydes and of coumarins for Spanish, French, and American wood samples after toasting are included in Table 2. Ferulic acid and scopoletin were not quantified because in all of the chromatograms of toasted woods, the peaks corresponding to these compounds were contaminated with another peak corresponding to an ellagic acid derivative. Without considering gallic and ellagic acids, which were major components also in toasted oak wood, phenolic aldehydes were the most abundant low molecular weight polyphenols in these wood extracts. The main component, in all species and origins, was coniferylic aldehyde, followed by, in decreasing order of abundance, sinapic, syringic, and vanillic aldehyde. Both benzoic acids, syringic and vanillic acid, showed lower concentrations, and aesculetin was a minor component.

Other nonphenolic components, derivatives of furfural, were detected in the HPLC chromatograms from organic extracts of toasted woods. 2-Furanoic acid and 5-(hydroxymethyl)furfural were quantified, although the method of extraction followed for the phenolic compounds was not the most suitable for their analysis (recovery was <50%). (Hydroxymethyl)furfural showed the highest concentration in ether extracts of the samples from all species and origins.

Considering the data obtained from Spanish toasted woods (Table 2) in relation to those of the nontoasted wood (Table 1), it can be pointed out that concentrations of phenolic compounds increased significantly during toasting (Table 3). Aldehydes showed the highest increment, especially cinnamic aldehydes, with average increases of 8900 and 4300% for sinapic and coniferylic
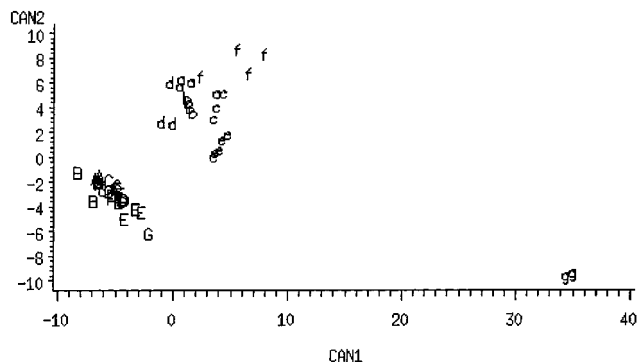
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Figure 3. Canonical discriminant analysis of low molecular weight polyphenols of seasoned and toasted woods; projections of the points of each species and provenance on the two principal canonical axes: (A) *Q. robur*, (B) *Q. petraea*, (C) *Q. pyrenaica*, and (D) *Q. faginea,* seasoned Spanish oak woods; (E) *Q. robur* and (F) *Q. petraea*, seasoned French oak woods; (G) *Q. alba*, seasoned American oak wood; (a) *Q. robur*, (b) *Q. petraea*, (c) *Q. pyrenaica*, and (d) *Q. faginea*, toasted Spanish oak woods; (e) *Q. robur* and (f) *Q. petraea*, toasted French oak woods; and (g) *Q. alba*, toasted American oak wood. Eigenvalues for discriminant functions 1 and 2 were 92.31 and 25.96, respectively. Standardized coefficients of discriminant functions 1 and 2 were as follows for each variable: gallic acid, 0.01 and 0.03; vanillic acid, −0.8 and 0.8; syringic acid, 8.8 and −5.3; ellagic acid, 0.04 and 0.27; vanillin, −4.2 and 6.6; syringaldehyde, −0.6 and −3.9; coniferaldehyde, 3.9 and 1.0; sinapaldehyde, 1.5 and 2.0; aesculetin, −0.5 and −0.3, respectively.

aldehydes, respectively, followed by benzoic aldehydes (syringaldehyde, 715%; and vanillin, 467%) and benzoic acids (syringic, 221%; and vanillic, 102%). The contents of ellagic and gallic acids and aesculetin did not experience significant variations in toasted wood with respect to nontoasted wood (Table 3). The canonical discriminant analysis carried out considering the wood samples before and after toasting provided a mathematical model that explained 100% of the total dispersion, distributed among nine canonical functions. The two principal canonical functions (1 and 2) presented a cumulative proportion of 95.7% of the total dispersion, canonical correlations of 0.995 and 0.981, and eigenvalues of 92.31 and 25.96, respectively. Figure 3 shows the graphical representation of the projections of the points of each group on the plane defined by functions 1 and 2. Phenolic aldehydes and syringic acid were selected as those that provided the greatest discrimination between nontoasted and toasted woods. Discriminant function 1 depended mainly on the content of syringic acid, vanillin, coniferylic aldehyde, and sinapic aldehyde, whereas function 2 depended mainly on the content of vanillin, syringic acid, syringic aldehyde, and sinapic aldehyde, in this sequence, as can be deduced from the standardized coefficients of discriminant functions 1 and 2, gathered in the legend of Figure 3. The graphical representation of the results of the discriminant analysis yielded a pattern of point distribution in which it is possible to distinguish two groups that are well separated and statistically very distant, corresponding to nontoasted and toasted woods. Moreover, there was more chemical variability among the samples of toasted woods than in nontoasted woods, according to the dispersion of the set of points shown in Figure 3.

To study the phenolic composition variability of toasted wood in Spanish species with respect to the French and American species, we carried out another discriminant analysis, considering only the wood samples
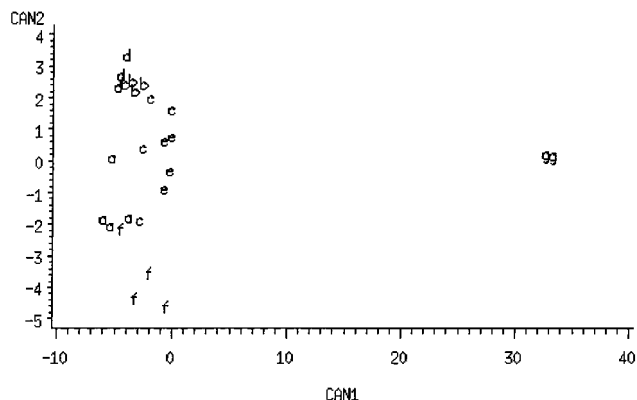

Figure 4. Canonical discriminant analysis of low molecular weight polyphenols of toasted oak woods, as shown by projections of the points of each species and provenance, on the two principal canonical axes: (a) *Q. robur*, (b) *Q. petraea*, (c) *Q. pyrenaica*, and (d) *Q. faginea*, from Spain; (e) *Q. robur* and (f) *Q. petraea*, from France; (g) *Q. alba*, from America. Eigenvalues for discriminant functions 1 and 2 were 134.66 and 6.14, respectively. Standardized coefficients for discriminant functions 1 and 2 were as follows for each variable: gallic acid, 0.03 and 0.5; vanillic acid, −1.1 and −0.7; syringic acid, 14.5 and 0.5; ellagic acid, −0.5 and −1.4; vanillin, −6.2 and −3.3; syringaldehyde, 0.2 and 4.9; coniferaldehyde, 1.6 and −1.1; sinapaldehyde, 0.3 and −1.5; aesculetin, 0.5 and 2.0, respectively.

after toasting. In this case the mathematical model resulting accounted for 100% of the total dispersion in six canonical functions, of which a cumulative proportion of 96% was represented on the plane determined by two principal canonical axes (Figure 4). The sets of points of Spanish species, especially *Q. pyrenaica*, overlapped with French *Q. robur* (Limousin) and separated from French *Q. petraea* (Allier), on the one hand, and from American *Q. alba*, on the other. The important differences of Spanish and French woods when compared with American wood were accounted for especially in canonical function 1, which showed as principal discriminant variables syringic acid and vanillin, whereas Spanish wood species and French *Q. robur* (Limousin) differed in canonical function 2, with syringic aldehyde and vanillin as principal discriminant variables (Figure 4).

DISCUSSION

During the 3 years of natural seasoning in the open air, the Spanish oak woods experienced a significant evolution of their chemical composition. The results indicate that the concentrations of most of the low molecular weight phenolic compounds increased during the seasoning process in the same way as in the first year of seasoning (*15*). We found a significant increase in some phenolic aldehydes such as vanillin, syringaldehyde, and coniferaldehyde, in accordance with previous studies on French and American oak woods (*13*, *31*). Also, the vanillic and syringic acids increased significantly, in accordance with the findings of Chatonnet et al. (*13*). The evolution of these concentrations was related to the seasoning duration. This effect was evidenced by Chatonnet et al. (*13*) in French oak wood (*Q. petraea*), in which they found a regular and constant increase of syringic and vanillic aldehyde contents during the first 3 years of natural seasoning, the syringaldehyde content being always higher than the vanillin one. The increment in the content of cinnamic

and benzoic acids and aldehydes in oak woods was explained by lignin degradation, by means of processes of depolymerization, and posterior hydrolytic and oxidative degradation of monomers, either by enzymatic (*46*) or by chemical (*25*, *47*) process.

The gallic and ellagic acid contents also increased during the seasoning stage but with different evolutions. Whereas the most important increase in gallic acid concentration happened during the first year of seasoning, in the case of ellagic acid this happened during the second and third years. Their origin was imputed to chemical hydrolysis and oxidation (*13*) and enzymatic degradation of ellagitannins (*13*, *28*, *48*). In this way, the decrease or disappearance of derivatives of ellagic and gallic acids could have contributed to the increase in these acids.

The discriminant analysis concerning the woods at the end of seasoning (Figure 2) distinguished the Spanish woods in four well-defined groups, with a pattern of distribution similar to that obtained in the same woods after 1 year of seasoning (*15*). The obtained groups showed a differentiation among species higher than in nonseasoned woods. The polyphenolic composition of Spanish oak woods varied according to species (*5*), but after seasoning this variation can be partially due to the way in which the wood samples respond to conditions of seasoning, which can differ considerably depending on the wood source (*11*, *12*, *15*).

Moreover, the polyphenolic composition of seasoned woods also permitted a differentiation of Spanish oak woods with respect to French and American woods, although statistically the chemical distance among the different species and origins was small, as can be deduced from the results of canonical discriminant analysis (Figure 2). *Q. robur* from France (Limousin) was the most different wood, as a result of its composition of phenolic aldehydes and acids.

Toasting was the process that most influenced the phenolic composition of wood. Toasting at medium intensity (35 min, 160−170 °C) was carried out to obtain high increases in the quantity of phenolic aldehydes and acids (Tables 1 and 2), without production of phenol type compounds (such as guaiacol, dimethoxyphenol, and cresol), which are characteristic of burned wood (*49*, *50*). These results are in accordance with previous studies of Sarni et al. (*20*) on the effect of heat treatment on the phenolic composition of oak wood, which revealed two phases in the process. The first one (120−165 °C) was characterized by the formation and increase of cinnamic aldehydes and benzoic aldehydes or acids, from the degradation of lignins (*37*), and the second one (165−195 °C) by the thermolysis of the aldehydes and acids into phenol type compounds (*16*, *20*). Our conditions of toasting would correspond to the first phase in which the aldehydes showed the highest increase, especially the cinnamic aldehydes (sinapic and coniferylic aldehydes), followed by benzoic aldehydes (syringaldehyde and vanillin) and benzoic acids (syringic and vanillic acids) (Tables 1 and 2), according to the mechanism: cinnamic aldehyde ⇒ benzoic aldehyde ⇒ benzoic acid, which involves the cleavage of α−β bonds of cinnamic aldehydes and the formation of benzoic acids (*20*).

At the beginning of toasting, there is a breakdown of celluloses that acts as the initiating stage in the degradation of lignins (*51*). The breaking of hydrogen and covalent bonds between oses and lignins destroys the cohesion of the lignocellulose network and facilitates the depolymerization by means of the rearrangement of the terminal units of lignin (*37*). The formation of cinnamic aldehydes can be a result of the breaking of aryl ether bonds (α-O-4 and β-O-4), which are less stable than C−C bonds (*20*).

The important increase in furanic derivatives, mainly 5-(hydroxymethyl)furfural and 2-furanoic acid, in the toasted oak wood with respect to the nontoasted one reflects the sugar degradation during toasting. The 5-(hydroxymethyl)furfural results from the degradation of aldohexoses of cellulose (*36*, *39*). Furanoic acid could come from pentoses of hemicelluloses, which, by thermodegradation, yield mainly furfural (2-furaldehyde) (*36*). These compounds or their derivatives and other derivatives of sugar degradation products such as pentacyclic and hexacyclic ketones (*22*, *23*) can play an important role in the aroma of barrel-aged wines.

Referring to the fact that ellagic and gallic acid contents do not experience significant variations in the toasted wood with respect to the nontoasted one (Table 3), our results were, basically, in line with those of previous studies. During wood toasting, we observed a decrease in the content of ellagitannins (*17*, *20*), which would produce ellagic and/or gallic acids by thermodegradation, but this process does not always go with an increase in the quantities of these acids, due to the fact that they can be sensitive to heat treatment.

Considering the results obtained on the phenolic composition of Spanish toasted wood (Table 2) with respect to those of seasoned (Table 1) and nonseasoned wood (*15*), it was very clear that toasting was the process in barrel cooperage that had the greatest influence on the chemical composition of wood, in accordance with the results obtained in French oak (*16*, *17*). It was also observed that phenolic compound quality and quantity are narrowly related to toasting intensity (*20*, *33*), but the particular characteristics of each species could determine the rate of modification in toasted wood. The chemical composition of Spanish, French, and American species evolved in the same way during toasting, but quantitative differences were observed, which led to a higher differentiation among the woods at the end of toasting. This differentiation was particularly important in the American species with respect to the others (Figure 4), possibly due to the fact that the staves from American oak were obtained by sawing, whereas those from Spanish and French oaks were obtained by cracking. This difference in the process of wood cutting could modify the physical properties of the staves and influence the response of wood to the toasting process.

As a consequence of the studies carried out to date on the low molecular polyphenolic composition of Spanish oak woods, we conclude that they can be considered to be suitable for barrel production.

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