Evolution of Phenolic Compounds of Spanish Oak Wood during Natural Seasoning. First Results

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Low molecular weight polyphenols and ellagitannins were analyzed by HPLC, and the molecular weight distribution of ellagitannins was calculated by GPC, in oak heartwood of *Quercus robur* L., *Quercus petraea* Liebl., *Quercus pyrenaica* Wild., and *Quercus faginea* Lam., grown in Spain, before and after 1 year of seasoning, in Bordeaux, France. During this process, the concentrations of low molecular weight polyphenols (acids and aldehydes, benzoic and cinnamic, and coumarins) increased, and those of ellagitannins (castalagin, vescalagin, and roburins A−E) decreased. A similar behavior for the A and B compounds in all species was not found. This modification in the chemical composition was similar in the four Spanish species of *Quercus* studied and allowed the differentiation between the unseasoned wood and the wood after the first year of seasoning.

Keywords: *Phenolic compounds; seasoning; wood; Quercus robur L.; Quercus petraea Liebl.; Quercus pyrenaica Wild.; Quercus faginea Lam.*

INTRODUCTION

In addition to the differences between species, the chemical composition of oak wood is also influenced by the geographical origin, the sylvocultural treatment of the tree, and the processing of wood in cooperage: how the staves are obtained, the method of seasoning, and the degree of oak toasting.

Seasoning produces the dehydration of wood, until its humidity rate is in balance with the ambient humidity. Regardless of climatic conditions and of thickness of wood (22−30 mm), the natural seasoning of wood for the cooperage is carried out for 10 or 12 months (Vivas and Glories, 1996). During this process, a certain contraction of fibers takes place and the wood humidity goes down from 75−60%, to a minimal value of 12% in dry regions, 18% in moist regions, and, in region of Bordeaux, usually around 15% (Vivas, 1993). This process has stages of dehydration, stages of rehumectation of the first millimeters of wood, and stages in the which the wood has a constant humidity (Vivas, 1994). The slowness of the process reduces the risk of the appearance of fissures in the staves.

However, in cooperage, the natural seasoning is much more than a stage in the dehydration of wood, necessary to keep together the pieces that make up a barrel. It is, basically, a refining stage, comparable to the slow and complex ripening of grapes (Vivas and Glories, 1993b). From being an unseasoned and aggressive wood, it becomes dry and aromatic, in which the soluble elements are smoother and pleasanter. Seasoning produces a loss of hydrosoluble substances, as the ellagitannins, and this contributes to a decrease of bitterness and astringency sensation (Marché and Joseph, 1975). This effect is particularly present in the surface of wood and to a lesser degree, but uniformly, in the inner wood. The decrease of hydrosoluble substances could be due to rain leaching of the staves. However, according to Vivas and Glories (1996), only between 0.035% and 0.07% of extractives of wood are removed by rain leaching. The decrease could be due also to a hydrolytic oxidative degradation process (Chatonnet et al., 1994b), with the formation of brown polymers (Klumpers and Janin, 1992; Klumpers et al., 1994) or to the insolubilization of oligomers ellagitanins after their polymerization (Peng et al., 1991; Klumpers et al., 1994).

Nevertheless, at the same time, on the surface and in the first millimeters of the wood staves, a important enzymatic activity (phenol heterosidase, etherase, and depsidase), of fungic nature, takes place (Joseph and Marché, 1972; Vivas et al., 1991, 1997). Although a large number of spores cover the surface of the wood after 6 months, only a few are able to develop a mycelium. The mycelium could penetrate the wood through the fissures and colonize the inner wood. In a study carried out by Vivas and Glories (1993a), more than 80% of the total fungi population isolated from wood staves during seasoning was *Aureobasidium pullulans*, and the main secondary species were *Trichoderma harzianum* and *Trichoderma konigii*. The polyphenolic profile of the wood is notably modified by this enzymatic activity, with glucose being released due to the destruction of heterosydic phenolic structures (coumarins and hydrolyzable tannins) and the resulting modification of organoleptic properties (decrease of astringency and bitterness) (Vivas et al., 1991; Vivas and Glories, 1993a). These biochemical reactions are influenced by physical mechanisms associated with pluviosity, UV radiation, and temperature variation (thermal amplitude) (Vivas and Glories, 1993b; Chatonnet et al., 1994b).

Besides organoleptic considerations, the elimination of the hydrosoluble polyphenols could have other consequences (Chatonnet, 1992). The decrease in the

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extractive fraction of these compounds during natural seasoning could influence the permeability of wood to gas, such as pine wood (Charuk and Razumova, 1974). Even the strip wood shrinkage during seasoning could be in relation to its content of hydrosoluble extractives, capable of taking up certain pores of the cell wall, which were full of water before seasoning (Monties, 1987).

In recent years, we have begun a study of the chemical characteristics of Spanish oak (Fernández de Simón et al., 1996a−c, 1998a,b). We have studied the polyphenolic composition of wood of four different species, grown in Spain, *Quercus robur*, *Q. petraea*, *Q. pyrenaica*, and *Q. faginea*, and we have compared these woods with others of the same species (*Q. robur* and *Q. petraea*) but of different origin (Limousin and Allier) and with samples of different species and origin (American *Q. alba*). In this work, we present the results obtained after the first year of natural seasoning, regarding the evolution of polyphenolic composition of Spanish oak wood.

EXPERIMENTAL PROCEDURE

Collection of Wood Samples. Heartwood of oak trees of *Q. robur* (seven trees), *Q. petraea* (three trees), *Q. pyrenaica* (five trees), and *Q. faginea* (three trees) grown in the Alava province (Spain) were provided by Centro Técnico de la Madera del PaísVasco, S. A. (Euskal Herriko Zuraren Teknica Bazkunea, S. A.). The wood was sent to Demptos Cooperage (Bordeaux, France) (average annual temperature = 12.5 °C; total precipitation = 950 mm/year; average over 50 years), where they proceeded to obtain the staves and carried out the process of natural seasoning in the open air that it will conclude in the next few months, after which the barrels will be made.

We have taken samples of heartwood, at the moment of being cut down and after 1 year of seasoning. The heartwood was processed into sawdust with a hammermill. The sawdust ranged in size from 0.75 to 0.28 mm.

Extraction. The sawdust samples (10 g) were extracted with 300 mL of methanol/water (1:1) at room temperature for 24 h. The extracts were filtered on a Büchner funnel and methanol was removed in a rotary evaporator, at a temperature below 40 °C. The aqueous solution was fractionated by liquid−liquid extraction with diethyl ether and ethyl acetate. The organic fractions were dried and redissolved in methanol. The remaining aqueous phase was freeze-dried.

Standards. Reference compounds were purchased from Fluka (gallic acid, aesculetin, and scopoletin), Aldrich (vanillic, sinapic, and ferulic acids, syringaldehyde, and coniferaldehyde), Apin (ellagic acid), Chem Service (syringic acid), Merck (vanillin), and Polymers Laboratories (polystyrene standards) and provided by Dr. Scalbert (castalagin, vescalagin, grandinin, roburin E, and roburin A).

GPC Analysis. The study of the MW distribution of tannins extracted from the heartwood was performed by using their acetyl derivatives. Samples (10 mg) of freeze-dried material were acetylated with pyridine−acetic anhydride (1:1.2) overnight at room temperature (Williams et al., 1983). The precipitate obtained by pouring this mixture into distilled water was recovered by vacuum filtration. The peracetate tannins were dissolved in tetrahydrofuran (THF) (2−5 mg/mL) and analyzed by gel permeation chromatography (Cadahía et al., 1996a; Fernández de Simón et al., 1996c). GPC analysis was performed using a HP−PL gel Mixed-D column, protected with a precolumn of the same material. The analysis conditions were THF as eluent, flow rate 1 mL/min, column temperature 30 °C, injection volume 20 μL, and analysis time 15 min. Detection was made at 255 nm with a bandwidth of 40 nm and at 280 nm with a bandwidth of 4 nm. The calibration curve was obtained with polystyrene standard. We also used acetylated standards of ellagic acid, vescalagin, and roburin A. The average molecular weight analyses were performed by using the Hewlett-Packard GPC software for HPLC Chemstation (Pascal Series).

HPLC Analysis of Low Molecular Weight Polyphenols. Samples of 20 μL of organic extract were analyzed by HPLC (Conde et al., 1995) in order to find the concentration of each phenolic compound. An apparatus with a diode array detector was used with a C_{18} Hypersyl ODS (5 μm) column (20 cm × 4 mm i.d.), protected with a precolumn of the same material. The elution conditions were 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 20% to 100% B, in 40 min; detection at 325 nm (with a bandwidth of 4 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 by the external standard method.

HPLC Analysis of Ellagitannins. Samples of 5 mg of freeze-dried material were dissolved in water and analyzed by HPLC (Scalbert et al., 1990; Cadahía et al., 1996b) in order to find the concentration of each ellagitannin. HPLC analysis was carried out in a chromatograph equipped with a diode array detector. The column used was a Hypersil ODS (200 × 4 mm i.d.), protected with a precolumn of the same material. Two solvents were used for elution: A = $MeOH/H_3PO_4$ (999:1) and B = H_2O/H_3PO_4 (999:1). The gradient profile was as follows: 0−40 min, 0−10% A; 40−70 min, 10−30% A; 70−90 min, 30−100% A. Chromatographic peaks were identified by comparing their retention time and their UV spectra with those of reference compounds. Roburin B, C, and D were identified by comparing the retention time and elution order with data described in the literature (Chatonnet et al., 1994b; Viriot et al., 1994; Vivas et al., 1996). Quantitative determinations were carried out by the external standard method. Roburins B−D were expressed as roburin A, since they are dimers.

Statistical Analysis. Data were analyzed by using the STATGRAPHICS program. Univariate analysis and discriminant canonical multivariate analysis were carried out. In univariate analysis, using a single-variable model, average, standard deviation, and coefficient of variation were calculated for each variable in each species. In discriminant canonical multivariate analysis, some mathematical functions called discriminant functions are calculated. On projecting the points of the euclidean space R^n and representing them graphically in two dimensions, the geometrical distances among the points are identical to the statistical distances.

RESULTS

In the HPLC analysis of diethyl ether and ethyl acetate extracts, the acids gallic, vanillic, syringic, ferulic, and ellagic, the aldehydes vanillic, syringic, coniferylic, and sinapic, and the coumarins aesculetin and scopoletin were identified in the four species of Spanish oak wood, before and after 1 year of natural seasoning (Figure 1). Table 1 reports their average concentrations and standard deviation. As can be seen, their concentrations were different according to the moment of seasoning and the species. In unseasoned wood, the main component was ellagic acid and the second was gallic acid. With respect to the remaining components, aldehyde contents were always higher than those of acids, and cinnamic compounds were more abundant than the benzoic ones, with the exception of ferulic acid, which was present in very low concentrations.

After 1 year of natural seasoning, only the average concentration of ferulic acid in *Q. petraea* decreased significantly. The rest of compounds increased their average concentrations during seasoning. The main component was gallic acid and the second was ellagic

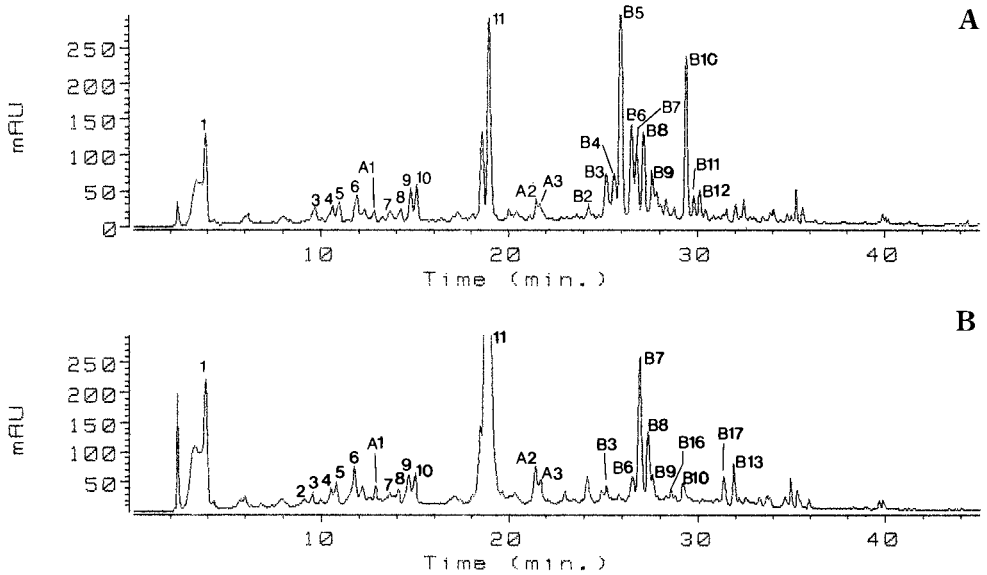

Figure 1. HPLC chromatograms (325 ± 75 nm) of ether extracts from heartwood of *Quercus* sp., before (A) and after seasoning (B) (1 = gallic acid; 2 = aesculetin; 3 = vanillic acid; 4 = syringic acid; 5 = vanillic aldehyde; 6 = syringaldehyde; 7 = scopoletin; 8 = ferulic acid; 9 = coniferaldehyde; 10 = sinapaldehyde; 11 = ellagic acid; A = A compounds; B = B compounds).

Table 1. Average (x) and Standard Deviation (sd) of Low Molecular Weight Phenolic Compounds, in Spanish Oak Wood before and after 1 Year of Natural Seasoning, Expressed as μG/g of Wood

	$x \pm$ sd before seasoning				$x \pm$ sd after 1 year of seasoning			
	Q. robur	*Q. petraea*	*Q. pyrenaica*	*Q. faginea*	*Q. robur*	*Q. petraea*	*Q. pyrenaica*	*Q. faginea*
acids								
gallic	100 ± 89	145 ± 75	63 ± 34	176 ± 52	341 ± 242	106 ± 31	489 ± 89	383 ± 89
vanillic	1.98 ± 0.63	1.90 ± 0.04	1.84 ± 0.63	1.72 ± 0.61	4.74 ± 3.31	4.32 ± 1.97	5.39 ± 0.30	2.65 ± 0.28
syringic	2.69 ± 1.85	2.31 ± 0.32	1.59 ± 0.44	1.66 ± 0.92	9.51 ± 7.85	6.97 ± 1.28	11.7 ± 1.33	6.39 ± 0.36
ferulic	1.30 ± 0.43	1.16 ± 0.78	0.51 ± 0.29	0.50 ± 0.26	1.06 ± 0.62	0.62 ± 0.45	0.72 ± 0.30	0.77 ± 0.06
ellagic	186 ± 36	195 ± 18	183 ± 41	213 ± 45	253 ± 27	224 ± 7.1	299 ± 60	340 ± 30
aldehydes								
vanillin	2.91 ± 1.20	2.77 ± 0.55	1.91 ± 0.99	1.54 ± 0.64	6.81 ± 4.52	5.94 ± 2.21	5.91 ± 0.70	3.76 ± 0.18
syringic	3.75 ± 1.56	3.55 ± 1.03	1.79 ± 0.81	2.03 ± 0.42	11.4 ± 5.08	8.15 ± 0.51	9.13 ± 0.67	5.75 ± 1.43
coniferylic	3.77 ± 0.53	3.85 ± 0.73	3.26 ± 1.45	4.26 ± 1.23	6.32 ± 3.41	5.07 ± 1.52	3.97 ± 1.92	3.95 ± 0.31
sinapic	3.94 ± 1.32	4.92 ± 0.53	2.48 ± 1.50	3.53 ± 1.93	4.29 ± 2.31	3.91 ± 2.79	5.11 ± 0.86	8.69 ± 0.33
coumarins								
aesculetin	1.83 ± 1.65	2.54 ± 1.77	0.83 ± 0.36	1.26 ± 0.97	5.62 ± 0.11	2.22 ± 0.44	2.65 ± 0.76	2.10 ± 0.18
scopoletin	2.27 ± 0.91	1.07 ± 0.54	2.04 ± 0.78	1.35 ± 0.69	2.84 ± 0.13	0.43 ± 0.51	6.46 ± 1.19	3.09 ± 0.91

acid, except in *Q. petraea*. Regarding aldehydes, their contents were always higher than those of acids, except syringaldehyde compared with syringic acid, in *Q. pyrenaica* and *Q. faginea*. Cinnamic compounds presented similar or lesser concentrations than the benzoic ones, although, in general, their average concentrations increased during natural seasoning.

On the whole, if we bear in mind the different species, before seasoning *Q. robur* and *Q. petraea* showed the highest amounts of these components, except for gallic and ellagic acids and coniferaldehyde, whose highest concentrations correspond to *Q. faginea*. After 1 year of natural seasoning, *Q. robur* and *Q. pyrenaica* were those that showed the highest amounts of these components, except for sinapic aldehyde and ellagic acid, whose highest concentrations correspond to *Q. faginea*. This species also showed higher amounts of gallic acid than *Q. robur*. The increases of average concentration were very important in *Q. pyrenaica*, especially in the case of gallic and syringic acid, whose average values became 7 times higher than before seasoning and in the case of syringic aldehyde became 5 times higher after 1 year of natural seasoning.

The discriminant canonical multivariate analysis of the concentrations of benzoic and cinnamic acids and aldehydes and coumarins provided two discriminant functions, which accounted for 89.53% of the total variance, with a canonical correlation of 0.9914 and 0.9759, respectively. Function 1 is made up of the main contribution of coniferaldehyde, ellagic acid, and syringaldehyde, in this sequence. This function distinguishes the four species, before and after seasoning. The function 2, made up of vanillic, syringic, and coniferylic aldehydes, differentiates *Q. robur* and *Q. petraea* from *Q. pyrenaica* and *Q. faginea*, before and after seasoning (Figure 2).

Moreover, two other types of low molecular weight phenolics have been found: A compounds, ellagic acid derivatives, and B compounds, gallic acid derivatives (Fernández de Simón et al., 1996a). Table 2 reports the variation in average concentrations and standard deviation of A and B compounds during seasoning. These variations differed according to compound and species.

In relation to A compounds, A-1, A-2, and A-3 were present before and after 1 year of seasoning. In *Q. pyrenaica* all increased their average values, whereas in the other species only one A compound increased: A-2 in *Q. robur*, A-3 in *Q. petraea*, and A-1 in *Q. faginea*. Regarding B compounds, there were differences between before and after the first year of seasoning and among species. There were some B compounds that were present in green wood and in seasoned wood decreased

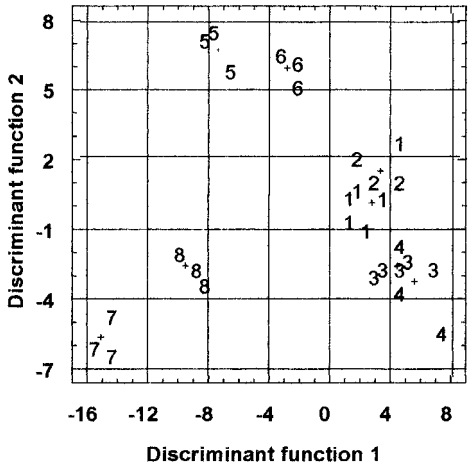

Figure 2. Multivariate analysis for benzoic and cinnamic acids and aldehydes and coumarins. Projections of the points of each species on the two principal canonical axes. 1, unseasoned *Q. robur*; 2, unseasoned *Q. petraea*; 3, unseasoned *Q. pyrenaica*; 4, unseasoned *Q. faginea*; 5, seasoned *Q. robur*; 6, seasoned *Q. petraea*; 7, seasoned *Q. pyrenaica*; 8, seasoned *Q. faginea*.

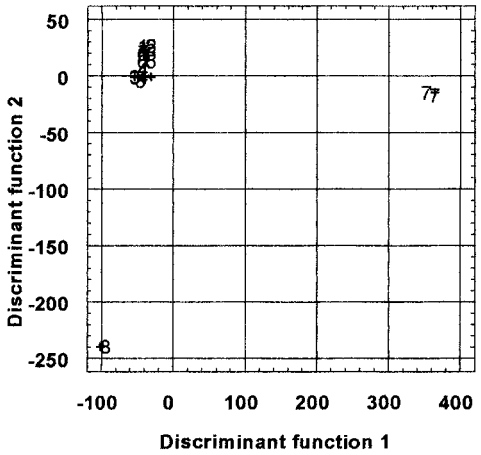

Figure 3. Multivariate analysis for B compounds. Projections of the points of each species on the two principal canonical axes. 1, unseasoned *Q. robur*; 2, unseasoned *Q. petraea*; 3, unseasoned *Q. pyrenaica*; 4, unseasoned *Q. faginea*; 5, seasoned *Q. robur*; 6, seasoned *Q. petraea*; 7, seasoned *Q. pyrenaica*; 8, seasoned *Q. faginea*.

their concentrations or were not found. There were other B compounds that were not found in green wood, but appeared in seasoned wood. The rest of the B compounds increased in concentration during seasoning. Moreover, each species has a different comportment during seasoning with respect to these compounds. The highest differences correspond to *Q. robur* and *Q. pyrenaica*. In *Q. robur*, most of the B compounds were not found after seasoning. Only two B compounds (B-8 and B-9) hardly varied, B-11 increased, and B-15 was found only after seasoning. In *Q. pyrenaica*, most of the B compounds increased their concentrations, except B-2, B-4, and B-9, which were not found after the first year of seasoning.

The discriminant canonical multivariate analysis of the concentrations of B compounds provided two discriminant functions, which accounted for 99.69% of the total variance, with a canonical correlation of 0.9999 and 0.9998. The graphical representation of the results, according to these discriminant functions, differentiates three groups of samples corresponding to seasoned *Q. faginea*, to seasoned *Q. pyrenaica*, and to the rest of the samples (Figure 3). Function 1 is made up of the main contribution of B-17, B-16, and B-14 compounds in this sequence. This function distinguishes the samples of seasoned *Q. pyrenaica* and seasoned *Q. faginea* from the rest of the samples.

In the GPC analysis (Figure 4), the tannins were eluted in three chromatographic peaks, corresponding to dimers (t_R = 8.56 min), monomers (t_R = 8.94 min), and ellagic acid (t_R = 9.6 min) (Fernández de Simón et al., 1998a,b). Almost all the tannic polymers were included in these three peaks.

The samples of different species and degree of seasoning presented similar chromatographic profiles, with small differences in the relative abundance of peaks. In all cases, the peak corresponding to monomers was the most important, followed by that of dimers, which, in some cases, appeared as a shoulder. The peak heights were used to determine the ratios of dimers to monomers (*D*/*M*) (Table 3): the average values in unseasoned

Table 2. Average (*x*) and Standard Deviation (sd) of A (Expressed as μg of Ellagic Acid/g of Wood) and B Compounds (Expressed as μg of Gallic Acid/g of Wood), in Spanish Oak Wood before and after 1 Year of Natural Seasoning[a]

		$x \pm$ sd before seasoning				$x \pm$ sd after 1 year of seasoning			
	t_r, min	*Q. robur*	*Q. petraea*	*Q. pyrenaica*	*Q. faginea*	*Q. robur*	*Q. petraea*	*Q. pyrenaica*	*Q. faginea*
A-1	12.7	9.6 ± 6.2	8.2 ± 3.0	10.1 ± 4.2	8.2 ± 5.7	7.3 ± 4.5	4.1 ± 1.3	15.0 ± 7.2	11.7 ± 3.5
A-2	21.3	24.5 ± 9.3	49.8 ± 20	25.1 ± 11.9	42.6 ± 29.6	45.9 ± 25.5	20.8 ± 6.7	60.5 ± 28.1	43.1 ± 0.32
A-3	21.6	14.7 ± 12.7	1.51 ± 2.59	1.78 ± 1.79	8.1 ± 10.0	8.28 ± 5.9	15.9 ± 3.7	6.7 ± 3.3	2.3 ± 0.96
B-1	23.5	39.6 ± 30.8	48.8 ± 17.6	11.2 ± 25.1				32.7 ± 11.6	
B-2	24.1	2.58 ± 3.31	0.89 ± 0.78						
B-3	25.0	4.60 ± 2.78	2.83 ± 2.45		5.52 ± 5.58		15.6 ± 12.2	32.8 ± 11.2	8.13 ± 8.06
B-4	25.4	2.95 ± 4.36	1.22 ± 0.18		3.62 ± 5.31				
B-5	25.8	23.5 ± 15.6	16.3 ± 14.0	18.3 ± 20.7	33.4 ± 29.6		3.35 ± 1.72	22.3 ± 2.77	
B-6	26.6	2.12 ± 3.04	0.48 ± 0.83	2.24 ± 2.51	4.07 ± 4.48			34.7 ± 3.76	9.64 ± 1.55
B-7	26.7	0.89 ± 0.79	0.63 ± 0.62	1.72 ± 0.94	8.19 ± 5.40			137 ± 7.09	108 ± 3.52
B-8	27.1	5.11 ± 6.91	16.1 ± 16.5	49.3 ± 8.96	45.0 ± 15.5	3.35 ± 1.71	5.64 ± 2.11	56.0 ± 8.28	24.6 ± 1.42
B-9	27.5	2.63 ± 2.83	1.29 ± 1.53	14.7 ± 6.08	6.61 ± 2.98	2.15 ± 3.73	7.12 ± 0.55		10.5 ± 3.24
B-10	29.3	30.1 ± 18.5	30.1 ± 13.5	55.0 ± 14.4	68.7 ± 14.4		4.02 ± 1.89	67.1 ± 17.3	8.60 ± 1.39
B-11	29.7	1.72 ± 1.12	0.73 ± 0.91	1.70 ± 1.48	1.30 ± 1.2	8.26 ± 4.53	6.35 ± 1.57	10.4 ± 2.98	
B-12	30.0	1.34 ± 0.88	1.61 ± 1.68	3.20 ± 1.90	3.57 ± 4.03		2.79 ± 0.26	13.7 ± 6.44	
B-13	32.1	0.62 ± 0.63	0.71 ± 0.62	10.6 ± 8.85	2.39 ± 0.92			50.9 ± 5.21	42.5 ± 0.05
B-14	22.4							39.3 ± 17.8	12.5 ± 3.60
B-15	28.0					1.75 ± 1.52	3.89 ± 1.73	47.1 ± 12.6	
B-16	28.5						1.75 ± 0.70	72.0 ± 11.5	79.1 ± 12.9
B-17	31.2							41.8 ± 6.27	26.8 ± 2.16

[a] A blank means not detected.

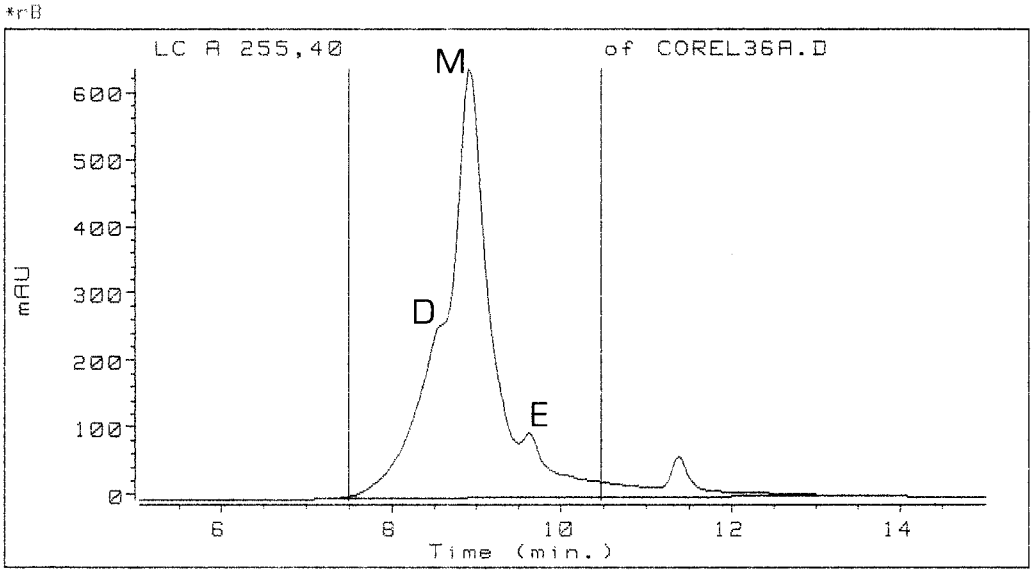

Figure 4. Chromatogram of acetylated tannins in heartwood extracts from *Quercus* sp. (detection at 255 nm; D = dimers; M = monomers; E = ellagic acid).

Table 3. Molecular Weights of Acetylated Tannins in Wood Extracts from Spanish *Quercus* spp., before and after 1 Year of Seasoning[a]

	$x \pm$ sd before seasoning				$x \pm$ sd after 1 year of seasoning			
	M_n	M_w	D_p	D/M	M_n	M_w	D_p	D/M
Q. robur	1419 ± 59	1958 ± 180	1.37 ± 0.06	0.49 ± 0.1	1067 ± 133	1453 ± 225	1.38 ± 0.050	0.47 ± 0.08
Q. petraea	1573 ± 119	2254 ± 250	1.42 ± 0.05	0.68 ± 0.07	970 ± 46	1354 ± 67	1.39 ± 0.005	0.57 ± 0.13
Q. pyrenaica	1512 ± 44	2228 ± 152	1.47 ± 0.07	0.56 ± 0.08	1131 ± 46	1641 ± 23	1.45 ± 0.020	0.54 ± 0.07
Q. faginea	1463 ± 129	2017 ± 257	1.37 ± 0.05	0.54 ± 0.09	1080 ± 48	1547 ± 78	1.43 ± 0.007	0.48 ± 0.11

[a] M_n = number-average molecular weight; M_w = weight-average molecular weight; D_p = polydispersity; x =average; sd = standard deviation; D = dimers; M = monomers.

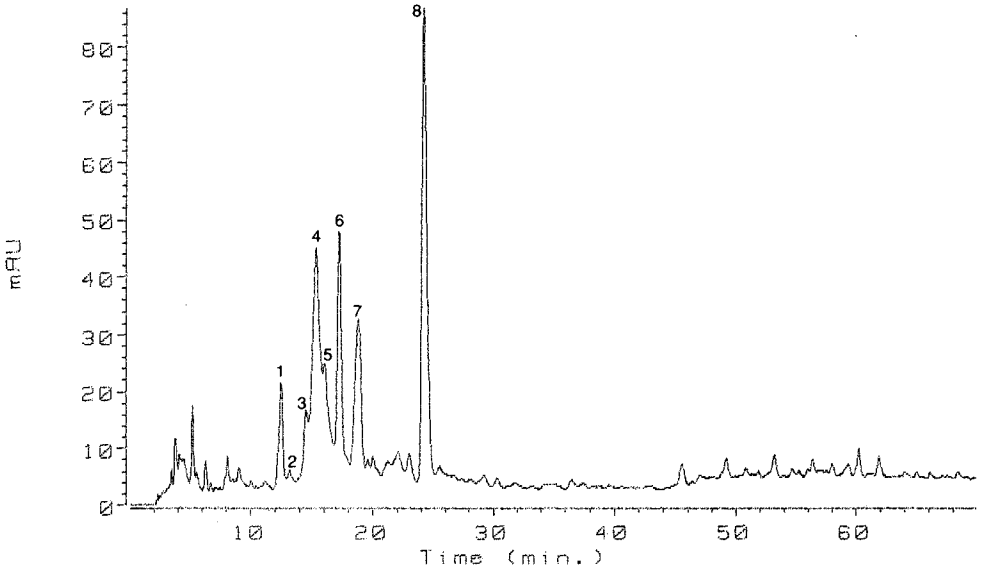

Figure 5. HPLC chromatogram (325 ± 75 nm) of acqueous extract from heartwood of *Quercus* sp. (1 = roburin A; 2 = roburin B; 3 = roburin C; 4 = grandinin; 5 = roburin D; 6 = vescalagin; 7 = roburin E; 8 = castalagin).

woods were higher than those of seasoned woods, but significant differences were not found. In the analysis of the molecular weight distribution of polymers, number-average molecular weight (M_n), weight-average molecular weight (M_w), and polydispersity ($D_p = M_w/M_n$) have been considered (Table 3). As can be seen, M_n and M_w decreased during seasoning, but in the same proportion, and therefore, D_p does not change. Significant differences were found for M_n and M_w in each species, before and after seasoning.

On the other hand, in the HPLC analysis of the tannic extracts from Spanish oak wood samples, we found ellagitannins monomers, such as castalagin, vescalagin, grandinin, and roburin E, and ellagitannins dimers, such as roburin A, B, C, and D (Figure 5). Every ellagitannin was found before and after the first year of seasoning. Therefore, the possible differences among samples were quantitative and not qualitative.

Table 4 reports the variation in average concentrations and the standard deviation of ellagitannins during

Table 4. Average (x) and Standard Deviation (sd) of Ellagitannins (mg/g of Wood), in Spanish Oak Wood before and after 1 Year of Seasoning

	$x \pm$ sd before seasoning				$x \pm$ sd after 1 year of seasoning			
	Q. robur	*Q. petraea*	*Q. pyrenaica*	*Q. faginea*	*Q. robur*	*Q. petraea*	*Q. pyrenaica*	*Q. faginea*
monomers								
castalagin	13.0 ± 4.32	9.06 ± 4.96	8.51 ± 4.41	10.1 ± 6.94	9.37 ± 2.18	6.97 ± 1.43	7.48 ± 0.50	9.67 ± 0.77
vescalagin	10.6 ± 3.78	8.01 ± 6.39	6.66 ± 3.60	7.01 ± 5.30	6.88 ± 8.01	1.84 ± 1.01	2.89 ± 1.31	6.66 ± 0.35
pentosylated monomers								
roburin E	9.20 ± 3.46	4.44 ± 1.62	4.78 ± 1.46	5.79 ± 5.54	6.13 ± 0.52	2.51 ± 0.20	3.21 ± 0.28	4.64 ± 0.27
grandinin	6.15 ± 2.6	5.47 ± 4.66	4.22 ± 1.86	5.43 ± 3.22	2.12 ± 1.28	0.75 ± 0.03	3.52 ± 0.24	4.13 ± 0.52
dimers								
roburin A	1.91 ± 0.95	0.98 ± 0.10	1.35 ± 0.77	0.97 ± 0.21	1.51 ± 0.40	0.43 ± 0.29	0.72 ± 0.23	0.20 ± 0.11
roburin D	0.95 ± 0.34	0.45 ± 0.23	0.51 ± 0.20	1.2 ± 0.85	0.69 ± 0.31	0.35 ± 0.09	0.62 ± 0.21	0.48 ± 0.33
pentosylated dimers								
roburin B	0.44 ± 0.27	0.46 ± 0.21	0.43 ± 0.22	0.47 ± 0.22	0.48 ± 0.13	0.31 ± 0.05	0.63 ± 0.14	0.64 ± 0.06
roburin C	1.72 ± 1.02	1.97 ± 1.07	1.66 ± 0.98	1.63 ± 0.87	1.27 ± 0.36	0.82 ± 0.07	0.68 ± 0.40	0.55 ± 0.31

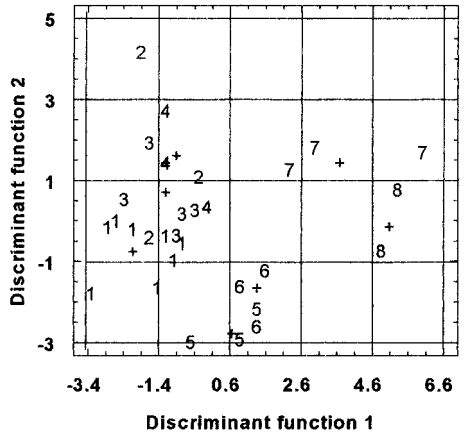

Figure 6. Multivariate analysis for ellagitannins. Projections of the points of each species on the two principal canonical axes. 1, unseasoned *Q. robur*; 2, unseasoned *Q. petraea*; 3, unseasoned *Q. pyrenaica*; 4, unseasoned *Q. faginea*; 5, seasoned *Q. robur*; 6, seasoned *Q. petraea*; 7, seasoned *Q. pyrenaica*; 8, seasoned *Q. faginea*.

seasoning. The most abundant ellagitannins in all the samples were the monomers, since their concentrations were much higher than those of the dimers. Castalagin shows the highest concentrations. All of them decreased their average concentrations during the first year of seasoning, in accordance with data in the literature (Chatonnet et al., 1994b). This decrease differs depending on the species and ellagitannin, and, therefore, a common behavior for monomers or dimers in the four species was not observed. In *Q. robur* and *Q. petraea,* grandinin was the ellagitannin with the highest decrease (75 and 86%, respectively), whereas roburin C (59%) and roburin A (79%) were the ellagitannins with the highest decreases in *Q. pyrenaica* and *Q. faginea,* respectively. In *Q. faginea* the dimers (except roburin B) decreased more than the monomers.

The discriminant canonical multivariate analysis of the concentrations of ellagitannins provided two discriminant functions which accounted for 76.45% of the total variance, with a canonical correlation of 0.9321 and 0.8458, respectively. The graphical representation of the results, according to these discriminant functions, allows us to differentiate the samples before and after seasoning (Figure 6). Function 1 is made up of the main contributions of roburin B, roburin C, and castalagin, in this sequence.

DISCUSSION

During natural seasoning in open air of oak wood for cooperage, the concentration of some components change remarkably, and a significant modification of oak wood chemical composition takes place. This evolution did not occur in the same way for all components studied: while the low molecular weight phenolic compounds increased their concentration during seasoning, the ellagitannins compounds, monomers and dimers, decreased. These results are in accordance with data in the literature (Chatonnet et al., 1994a,b; Swan et al., 1993).

In regard to low molecular weight polyphenols, Sefton et al. (1993a) observe that neither oak origin, seasoning period, nor location of seasoning appeared to have a major influence on the vanillin content of oak wood. On the contrary, Swan et al. (1993) point to a significant increase of the content of vanillin, syringaldehyde, and sinapaldehyde. In the same way, these same authors detect a regular degradation of lignin during the aging wood period. Moreover, Chatonnet et al. (1994c) found during seasoning of staves a net increase of vanillin and syringaldehyde and, especially, of dimethoxylated compounds (syringaldehyde). Our results showed not only an increase of vanillin, syringaldehyde, coniferaldehyde, and sinapaldehyde but also of vanillic and syringic acids. This increase was higher for syringic acid and aldehyde (dimethoxylated) than for vanillic acid and aldehyde (monomethoxylated), according to Chatonnet et al. (1994c). As can be seen in Figure 2, the evolution of the concentrations of these compounds during seasoning allows to distinguish between unseasoned and seasoned wood. Moreover, the statistical distances among species increases with seasoning.

The presence of these compounds in oak woods is due to a lignin degradation process. The lignin is a tridimensional polymer made up of two phenylpropanoide alcohols: 4-hydroxy-3-methoxycinnamic alcohol (coniferylic) and 4-hydroxy-3,4-dimethoxycinnamic alcohol (sinapilic). The cinnamic and benzoic acids and aldehydes are formed from these two alcohols, by hydrolytic and oxidative processes. In regard to enzimatic degradation of lignin, Vivas and Glories (1996) show that neither the different bacteria nor fungi isolated seem to be able to degrade the lignin, according to the work of Chatonnet et al. (1994a). So, the process of depolymerization of lignin and the posterior hydrolytic and oxidative degradation of monomers ought to be an effect of the combined presence of water and oxygen that, during natural seasoning, is produced; that is to say, it is a chemical process (Monties, 1992).

In regard to coumarins aesculetin and scopoletin, their average concentration increased during seasoning. This increase is fully explained in the literature (Joseph and Marché, 1972; Chatonnet et al., 1994b; Vivas and Glories, 1996), since while their glycosides (aesculin,

scopolin) are very bitter, the aglycones are more neutral, in gustative plain, and this could be directly linked to the loss of bitterness and astringency of wood during seasoning (Marché and Joseph, 1975; Taransaud, 1976; Sefton et al., 1993b; Vivas, 1994).

Gallic and ellagic acids also increased their concentrations during seasoning, specially gallic acid. Although it is not clear what their origin is, they can come from enzimatic degradation of phenolic heterosides (Vivas, 1993), since the different fungus species isolated are able to develop a micelium in liquid medium, using the ellagitannins in solution as a carbon source (Vivas et al., 1996).

On the other hand, the decrease of oligomer ellagitannins in wood during seasoning can have different origins. In the first place, it is necessary to take into account that they are hydrosoluble compounds and can be carried away by rainwater in leaching, but at a slow rate (Vivas and Glories, 1996). Moreover, the ellagitannins are molecules sensitive to chemical hydrolysis and oxidation (Chatonnet et al., 1994b), as well as to enzimatic degradation produced by fungi (Vivas et al., 1991; Vivas, 1993; Chatonnet et al., 1994b).

Chatonnet et al. (1994b) found a higher sensitivity to chemical and enzimatic degradation in the dimers than in the monomers. However, we did not find a common behavior for dimers and monomers in the four species of *Quercus* studied. Although the D/M rate decreased during seasoning, in GPC analysis, significant differences were not found. Only in *Q. faginea* the dimers decreased more than the monomers, except for roburin B. As it happened with low molecular weight polyphenols, the evolution of the concentrations of these compounds during seasoning allows one to distinguish between unseasoned and seasoned wood. Moreover, the statistical distances among species increased with seasoning.

If we bear in mind the results obtained on the whole, the modification of the chemical composition produced in oak wood with seasoning was the same in the four species studied. However, the way in which the *Quercus* sp fitted to the process was different and allowed a better differentiation among species at the end of seasoning. Specific characteristics of each species, as porosity, could determine the rate at which this substances can be leached, the rate of penetration of oxygen and water, and the rate of degradation by microflora.

CONCLUSION

The natural seasoning of the oak wood process is something more that a simple dehydration process, it is more similar to a maturation process in which the wood modifies its chemical composition, along with its physical properties. This chemical composition modification was similar in the four Spanish species of *Quercus* studied and allowed us to distinguish between the unseasoned wood and the wood after its first year of seasoning.

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