Evolution of Ellagitannins in Spanish, French, and American Oak Woods during Natural Seasoning and Toasting

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The evolution of tannins in Spanish oak heartwood of *Quercus robur* L., *Quercus petraea* Liebl., *Quercus pyrenaica* Wild*.,* and *Quercus faginea* Lam*.* was studied in relation to the processing of wood in barrel cooperage. Their evolution was compared with that of French oak of *Q. robur* (Limousin, France) and *Q. petraea* (Allier, France) and American oak of *Quercus alba* L. (Missouri), which are habitually used in cooperage. Two stages of process were researched: the seasoning of woods during 3 years in natural conditions and toasting. Total phenol and total ellagitannin contents and optical density at 420 nm of wood extracts were determined. The ellagitannins roburins A−E, grandinin, vescalagin, and castalagin were identified and quantified by HPLC, and the molecular weight distribution of ellagitannins was calculated by GPC. During the seasoning process the different ellagitannin concentrations decreased according to the duration of this process and in the same way as those in French and American woods. The toasting process also had an important influence on the ellagitannin composition of wood. Roburins A−E, grandinin, vescalagin, and castalagin decreased during this process in the Spanish wood species, in the same proportion as in the French and American ones. Also, the seasoning and toasting processes lead to qualitative variations in the structure of ellagitannins, especially in the molecular weight distribution, as was evidenced by GPC analysis of their acetylated derivatives.

INTRODUCTION

The heartwood of oak species, traditionally used in cooperage for making barrels, contains high concentrations of phenolic extractives, mainly ellagitannins. The ellagitannins identified from oak wood were castalagin, vescalagin (*1, 2*), grandinin, and roburins A−E (*3−5*). These tannins are hydrosoluble and, when dissolved in hydroalcoholic mediums such as wines (*6, 7*), they have oxidizing potential (*8, 9*) and taste properties (*9*) that give them a potential role in the aging process in oak barrels (*10, 11*).

The polyphenol concentration, including ellagitannins, extracted from wood into the wine during its aging depends on the pool of these compounds present in the barrel wood. Their structures and amounts can be affected by the species and other factors such as geographical origin (*11−16*), but during barrel production, the oak wood used in cooperage must pass through several processing stages, which are important to the wood composition. Especially, the seasoning and toasting processes play critical roles in the tannic composition of oak wood (*16−18*).

Seasoning results in the dehydration of wood, until its humidity rate is in line with the ambient humidity. However, in cooperage, natural seasoning is much more than a stage in the dehydration of wood, necessary to keep the pieces that make up a barrel together. It is, basically, a refining stage, comparable to the slow and complex ripening of the grapes, through which unseasoned and aggressive wood becomes dry and aromatic (*19*).

During the seasoning process there is a loss of hydrosoluble polyphenolic substances, such as the ellagitannins, and this contributes to a decrease in the sensation of bitterness and astringency (*20, 21*). The effect is particularly important on the surface of wood and, to a lesser degree, but uniformly, in the inner wood. Different mechanisms could be involved in this process: the rain leaching of the staves (*22*), hydrolytic oxidative degradation process (*21, 23*) with the formation of brown polymers (*24*), precipitation of oligomeric ellagitannins after their polymerization (*24, 25*), and also enzymatic activity (phenol heterosidase, etherase, and depsidase) of fungic nature (*26*) with the destruction of heterosydic phenolic structures, such as coumarins and hydrolyzable tannins. These biochemical reactions are influenced by physical mechanisms associated with rainfall, UV radiation, and temperature variation (*19, 21*).

The elimination of the hydrosoluble polyphenols during natural seasoning could also have other consequences. It could influence the permeability of wood to gas. Even the strip wood shrinkage during seasoning could be related to the content of hydrosoluble extractives, capable of taking up certain pores of the cell wall, which were full of water before seasoning (*27*).

However, the most significant change in the chemical composition of wood probably takes place during the toasting process in barrel production, affecting the organoleptic properties of wine. The heat treatment of

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oak staves is a technique necessary to facilitate stave assembly. Both the quality and quantity of the oak wood extractable substances vary with the toasting level; therefore, mastery and repeatability are vital in quality barrel production (*28–30*).

Concerning the tannic composition of wood, heat treatment also results in a decrease in the amount of ellagitannins (castalin and castalagin) and a simultaneous increase in ellagic acid, according to the toasting conditions (*17, 30, 31*).

Over the past few years we have been carrying out extensive research on the chemical characteristics of Spanish oak for use in cooperage. 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 different origin (Limousin and Allier) and with samples of different species and origin (American *Quercus alba*) (*15, 32–35*). We have also studied the evolution of the low molecular weight phenolic compounds of Spanish oak wood during natural seasoning and toasting (*18, 36, 37*). In this work, we present the results obtained on the evolution of the tannic composition of Spanish oak wood during 3 years of natural seasoning and toasting in relation to French and American oak woods used in barrel production.

MATERIALS AND METHODS

Wood Sample Collection. Heartwood samples of *Q. robur* (seven trees), *Q. petraea* (three trees), *Q. pyrenaica* (five trees), and *Q. faginea* (three trees) grown in the Álava 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, carry 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 a 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 (*38*). 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. These staves were also toasted according to the same process (*38*).

Wood samples were taken from the nontoasted and toasted staves, four 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 toasted stave pieces, 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, following the method described by Fernández de Simón et al. (*35*). The aqueous solution (without methanol) was used for the quantitative determination of the Folin–Ciocalteu index and optical density (OD). This solution was extracted with diethyl ether and ethyl acetate and freeze-dried, and this material was used for HPLC qualitative and quantitative analysis and for the GPC analysis of ellagitannins.

Standards. Standards of vescalagin, castalagin, roburins A and E, and grandinin were kindly provided by Dr. A. Scalbert. Polystyrene standards from Polymer Laboratories with molecular weights in the range 162–22000 were used for GPC analysis.

Analytical Methods. The Folin–Ciocalteu index was estimated following the classic method (*39*), and ellagitannins were determined by analysis by HPLC of the ellagic acid yielded after they were submitted to methanolysis (*36*). The optical density at 420 nm was also determined.

HPLC Analysis. HPLC analysis of ellagitannins was carried out according to the method of Cadahía et al. (*40*). 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 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); gradient profile, 0–10% B in 0–40 min, 10–30% B in 40–70 min, 30–100% B in 70–90 min; detection at 325 nm (with a bandwidth of 150 nm). Quantitative determinations were carried out according to the external standard method. Roburins B–D were expressed as roburin A because they are also dimers.

GPC Analysis. The molecular weight distribution analysis of tannins was performed on their acetyl derivatives by using gel permeation chromatography, according to the method of Cadahía et al. (*41*). In this case the chromatograph was equipped with an HP-PL gel Mixed-D column and THF was used as eluent (flow rate = 1 mL/min). Detection was made 270 nm with a bandwidth of 30 nm.

Statistical Analysis. Univariate analysis was carried out, using the BMDP-7D (ANOVA) program (W. J. 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

Variation of Oak Wood Composition during Natural Seasoning. The results of the chemical analysis of the oak wood subjected to 3 years of natural seasoning (nontoasted woods), gathered in Table 1, showed higher extractable component contents (Folin–Ciocalteu index) in the Spanish wood species than in the French *Q. petraea* and American *Q. alba*, similar to those of French *Q. robur*. However, the differences were significant only for Spanish *Q. petraea* and *Q. faginea* with respect to the French *Q. petraea*, due to the high variability into the species and origins. Total ellagitannins showed behavior similar to that of total phenols. This indicates that the Folin–Ciocalteu index in the oak woods studied varies depending on the high molecular weight polyphenols (ellagitannins), because they are the water-soluble polyphenols presented in the heartwood (*7, 42*). The optical density at 420 nm of extracts from the Spanish oak was also similar to those of the French oak *Q. robur* and the American *Q. alba*, which indicates a similar degree of oxidation of the polyphenols in the different extracts.

Individual analysis, by HPLC, of extractable ellagitannins from Spanish seasoned oak woods showed quantitative and not qualitative differences with respect to the French and American oaks (Table 2). The most abundant ellagitannins in all of the species were the monomers, as was proved in the same Spanish woods before seasoning (*15*); however, in this case, the decreasing order of abundance was castalagin, roburin E, vescalagin, and grandinin. Considering the global average of each ellagitannin, it can be pointed out that, in

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Table 1. Optical Density (OD) at 420 nm, Folin–Ciocalteu Index, and Ellagitannin Content of Spanish, French, and American Oak Wood Extracts, after 3 Years of Natural Seasoning and after Toasting[a]

	OD at 420 nm	Folin–Ciocalteu index (mg of gallic acid/g of wood)	ellagitannins (mg of ellagic acid/g of wood)
Nontoasted			
Spanish oak			
Q. robur	0.24 ± 0.05 ab	42.6 ± 6.22 ab	8.24 ± 1.41 c
Q. petraea	0.21 ± 0.05 a	53.7 ± 7.22 b	12.1 ± 1.77 d
Q. pyrenaica	0.22 ± 0.03 a	44.8 ± 7.79 ab	5.86 ± 2.75 bc
Q. faginea	0.11 ± 0.01 a	50.5 ± 11.1 b	8.52 ± 1.28 c
French oak			
Q. robur	0.21 ± 0.02 a	45.4 ± 14.6 ab	6.90 ± 3.44 bc
Q. petraea	0.38 ± 0.08 bc	25.7 ± 5.87 a	4.71 ± 0.95 abc
American oak			
Q. alba	0.23 ± 0.01 ab	30.7 ± 0.98 ab	4.06 ± 0.24 abc
Toasted			
Spanish oak			
Q. robur	0.54 ± 0.13 d	37.6 ± 12.4 ab	5.01 ± 2.13 abc
Q. petraea	0.55 ± 0.05 d	46.5 ± 10.0 ab	5.99 ± 0.72 bc
Q. pyrenaica	0.54 ± 0.10 d	42.2 ± 13.2 ab	4.79 ± 1.83 abc
Q. faginea	0.38 ± 0.08 bc	40.6 ± 2.53 ab	5.21 ± 0.70 abc
French oak			
Q. robur	0.60 ± 0.11 d	52.4 ± 13.3 b	7.79 ± 1.73 c
Q. petraea	0.46 ± 0.04 cd	28.8 ± 0.99 ab	2.76 ± 0.78 ab
American oak			
Q. alba	0.64 ± 0.01 d	27.6 ± 3.85 ab	0.34 ± 0.20 a

[a] Average and standard deviation ($x \pm$ SD) were calculated for four samples in Spanish and French oak and for two samples in American oak. Different letters denote a significant difference with 95% confidence level in the Student–Newman–Keuls multiple-range test.

general, the Spanish seasoned woods presented higher values than the French *Q. petraea* and the American *Q. alba* and values similar to those of the French *Q. robur*. The Spanish *Q. petraea* was the wood that presented the highest concentrations of all ellagitannins, in accordance with the results obtained of total phenol and total ellagitannin contents (Table 1). However, we must emphasize the important variability presented by most of the variables in all of the woods analyzed, which can be responsible for the few or unremarkable significant differences among species and origins, according to variance analyses (Table 2). The

exception was the Spanish *Q. petraea*, which showed significant differences with respect to the other woods in the concentrations of castalagin, vescalagin, roburin E, and roburin A (Table 2).

In relation to the variation of the ellagitannin composition throughout the seasoning process, if we compare the results obtained from the Spanish woods seasoned during 3 years (Tables 1 and 2) with those obtained from the same woods but before seasoning and after only 1 year of seasoning (*15, 18*), they reveal a great decrease in the average contents of the monomeric ellagitannins, especially castalagin and vescalagin. This decrease was more important during the first stage of seasoning. The mean contents in Spanish oaks (average of *Q. robur, Q. petraea, Q. pyrenaica,* and *Q. faginea*) before seasoning, after 1 year of seasoning (*18*), and after 3 years of seasoning (Table 2) were, respectively, the following: 10.2, 8.37, and 7.98 mg/g for castalagin and 8.07, 4.57, and 4.25 mg/g for vescalagin. The optical density and Folin–Ciocalteu index also decreased during seasoning, with the mean values going from 0.69 to 0.19 and from 73.6 to 47.9 mg/g, respectively (Table 1; *15*).

GPC analysis of seasoned Spanish woods (Table 3) showed chromatographic profiles similar to those obtained from the same woods before seasoning and after 1 year of seasoning (*15, 18*). The tannins were also 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), but in this case the proportion of the peak corresponding to monomers with respect to dimers varied. The four Spanish species showed the same type of molecular weight distribution curve, with similar average values of M_n (number-average molecular weight), M_w (weight-average molecular weight), Dp (polydispersity) and D/M (dimers/monomers), and unremarkable significant differences among species were observed in the variance analyses (Table 3). The differences were not significant with respect to the French and American seasoned oak woods. If we compare these results (Table 3) with those corresponding to nonseasoned and 1-year-seasoned

Table 2. HPLC Quantitative Evaluation of Ellagitannins (Milligrams per Gram of Wood) in Spanish, French, and American Oak Wood Extracts, after 3 Years of Natural Seasoning and after Toasting[a]

	monomers		pentosylated monomers		dimers		pentosylated dimers	
	castalagin	vescalagin	roburin E	grandinin	roburin A	roburin D	roburin B	roburin C
Nontoasted								
Spanish oak								
Q. robur	6.68 ± 1.77 bc	4.62 ± 2.53 a	5.26 ± 1.56 cd	3.69 ± 1.05 cd	1.09 ± 0.25 abc	2.52 ± 0.93 c	0.26 ± 0.13 bc	0.24 ± 0.10 e
Q. petraea	12.5 ± 2.07 d	7.96 ± 4.16 b	7.98 ± 0.55 e	4.83 ± 0.98 d	2.43 ± 0.67 d	2.58 ± 0.24 c	0.32 ± 0.04 c	0.21 ± 0.02 de
Q. pyrenaica	4.54 ± 2.91 abc	1.68 ± 1.07 a	3.87 ± 1.95 bcd	3.10 ± 1.52 bc	0.73 ± 0.29 ab	1.74 ± 0.80 bc	0.28 ± 0.09 c	0.16 ± 0.06 cde
Q. faginea	8.18 ± 2.43 c	2.76 ± 0.40 a	5.85 ± 1.46 d	3.21 ± 1.25 bc	1.51 ± 0.45 c	2.12 ± 0.77 c	0.31 ± 0.04 c	0.17 ± 0.04 cde
French oak								
Q. robur	6.11 ± 3.94 bc	5.00 ± 3.86 a	3.51 ± 2.53 abc	2.03 ± 1.57 abc	1.27 ± 0.92 bc	1.02 ± 0.81 ab	0.18 ± 0.09 abc	0.14 ± 0.05 cde
Q. petraea	3.43 ± 1.76 abc	1.85 ± 1.69 a	2.74 ± 0.96 ab	1.81 ± 0.54 ab	0.50 ± 0.36 ab	1.07 ± 0.32 ab	0.15 ± 0.04 abc	− a
American oak								
Q. alba	2.86 ± 0.04 abc	1.14 ± 0.39 a	1.75 ± 0.31 ab	1.05 ± 0.28 a	0.23 ± 0.05 ab	0.84 ± 0.23 ab	0.12 ± 0.01 abc	− a
Toasted								
Spanish oak								
Q. robur	3.60 ± 1.37 abc	0.89 ± 0.17 a	1.80 ± 0.91 ab	0.28 ± 0.21 a	0.41 ± 0.22 ab	0.45 ± 0.33 a	0.17 ± 0.11 abc	0.12 ± 0.04 cd
Q. petraea	3.79 ± 1.03 abc	1.02 ± 0.75 a	2.23 ± 0.28 ab	0.59 ± 0.06 a	0.50 ± 0.14 ab	0.44 ± 0.09 a	0.22 ± 0.04 bc	0.17 ± 0.02 cde
Q. pyrenaica	2.77 ± 0.93 ab	0.37 ± 0.34 a	1.66 ± 0.79 ab	0.42 ± 0.28 a	0.41 ± 0.18 ab	0.38 ± 0.16 a	0.21 ± 0.07 bc	0.15 ± 0.04 cde
Q. faginea	4.68 ± 0.67 abc	0.96 ± 0.42 a	2.27 ± 0.30 ab	− a	0.24 ± 0.21 ab	0.80 ± 0.23 ab	0.25 ± 0.04 bc	0.14 ± 0.03 cde
French oak								
Q. robur	5.44 ± 2.56 abc	1.15 ± 0.64 a	2.39 ± 0.80 ab	0.66 ± 0.21 a	0.47 ± 0.12 ab	0.50 ± 0.16 a	0.20 ± 0.01 bc	0.19 ± 0.02 de
Q. petraea	1.75 ± 0.49 ab	0.36 ± 0.08 a	0.75 ± 0.17 a	0.13 ± 0.03 a	0.20 ± 0.06 a	0.16 ± 0.03 a	0.10 ± 0.02 ab	0.08 ± 0.02 bc
American oak								
Q. alba	− a	− a	− a	− a	− a	− a	− a	− a

[a] Average and standard deviation ($x \pm$ SD) were calculated for four samples in Spanish and French oak and for two samples in American oak. Different letters denote a significant difference with 95% confidence level in the Student–Newman–Keuls multiple-range test.

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Table 3. Molecular Weights of Acetylated Tannins in Wood Extracts from Spanish, French, and American Oaks, after 3 Years of Natural Seasoning and after Toasting[a]

	M_n[b]	M_w[c]	Dp[d]	D/M[e]
		Nontoasted		
Spanish oak				
Q. robur	1586 ± 132 abc	2523 ± 280 abc	1.58 ± 0.06 bc	0.85 ± 0.21 a
Q. petraea	1441 ± 20 ab	2113 ± 54 a	1.46 ± 0.03 a	0.76 ± 0.18 a
Q. pyrenaica	1597 ± 104 abc	2553 ± 204 abc	1.60 ± 0.03 bc	0.98 ± 0.12 a
Q. faginea	1433 ± 149 ab	2209 ± 240 a	1.54 ± 0.02 ab	0.90 ± 0.12 a
French oak				
Q. robur	1419 ± 46 a	2199 ± 213 a	1.55 ± 0.11 ab	0.81 ± 0.16 a
Q. petraea	1690 ± 10 bc	2725 ± 23 bc	1.61 ± 0.01 bc	0.77 ± 0.06 a
American oak				
Q. alba	1516 ± 65 abc	2473 ± 127 abc	1.63 ± 0.01 bc	0.87 ± 0.11 a
		Toasted		
Spanish oak				
Q. robur	1669 ± 34 abc	2730 ± 112 bc	1.63 ± 0.05 bc	1.06 ± 0.09 a
Q. petraea	1519 ± 118 abc	2328 ± 191 bc	1.53 ± 0.01 ab	0.89 ± 0.21 a
Q. pyrenaica	1672 ± 98 bc	2735 ± 225 bc	1.63 ± 0.04 bc	1.06 ± 0.09 a
Q. faginea	1453 ± 216 ab	2454 ± 269 abc	1.70 ± 0.07 c	0.83 ± 0.05 a
French oak				
Q. robur	1746 ± 55 c	2847 ± 125 c	1.63 ± 0.02 bc	1.01 ± 0.03 a
Q. petraea	1699 ± 30 bc	2650 ± 81 abc	1.56 ± 0.02 ab	0.88 ± 0.03 a
American oak				
Q. alba	1609 ± 92 abc	2673 ± 167abc	1.66 ± 0.01 bc	1.27 ± 0.01 a

[a] Average and standard deviation ($x ±$ SD) were calculated for four samples in Spanish and French oak and for two samples in American oak. Different letters denote a significant difference with 95% confidence level in the Student−Newman-Keuls multiple-range test. [b] M_n = number-average molecular weight. [c] M_w = weight-average molecular weight. [d] Dp = polydispersity. [e] D/M = dimers/monomers.

Spanish woods (*18*), an increase in the dimer proportion with respect to the monomers can be observed during the second stage of seasoning (averages D/M for non-seasoned, 1-year-seasoned and 3-year-seasoned Spanish woods were 0.57, 0.51, and 0.87, respectively). This increase is also reflected in the average values of M_n and M_w.

Variation of Oak Wood Composition during Toasting. The chemical analysis (Table 1) carried out on the toasted oak woods showed a similar behavior in the Spanish species with respect to the French and American species. The Folin−Ciocalteu index and total ellagitannin values of extracts from all of the Spanish woods studied were found to be between those obtained from the French *Q. petraea*, the American *Q. alba*, and the French *Q. robur*, although the variance analysis did not show significant differences due to the high variability into the species and origins. The optical density values at 420 nm of Spanish extracts were also halfway between those of the French *Q. petraea* and the *Q. robur*, the latter ones being similar to those of the American *Q. alba.*
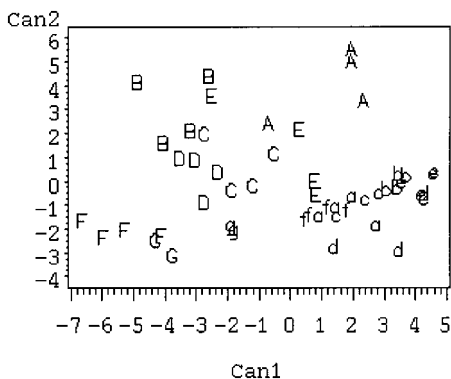
Considering the data obtained from the Spanish toasted woods in relation to those of the nontoasted woods (Table 1), it can be seen that ellagitannin contents showed a decrease during toasting and that the Folin−Ciocalteu index remained constant. However, the optical density increased significantly in all of the woods after toasting.

The HPLC analysis of the tannic extracts from toasted woods revealed the presence of the same ellagitannins identified in the woods before toasting (Table 2). The most abundant ellagitannins were also the monomers, which showed the same decreasing order of abundance as in the seasoned woods: castalagin, roburin E, vescalagin, and grandinin. Considering the data obtained from the toasted woods in relation to those of nontoasted woods, it can be pointed out that, in general, the concentrations of the monomeric and dimeric ellagitannins in the Spanish species decreased during toasting, in the same proportion as in the French species and the

American *Q. alba*, in which appreciable quantities of each ellagitannin were not found. The decrease was particularly important in the case of monomers and pentosylated monomers, even though it was significant only in the latter, according to the variance analysis gathered in the same Table 2, due to the high variability shown by different samples into the species and origins. The concentrations of each ellagitannin present in the Spanish toasted woods were found to be halfway between those the French *Q. petraea*, the American *Q. alba*, and the French *Q. robur*.

GPC analysis, gathered in Table 3, did not show important differences in the molecular weight distribution curves for the toasted woods with respect to the nontoasted woods. A tendency toward increasing values of M_n, M_w, and D/M during toasting can therefore be deduced, but the differences found in the analysis of variance were not significant, possibly due to high variability into species and provenance.

To compare the different species and origins of woods at the end of seasoning and toasting, we carried out a canonical discriminant analysis considering the ellagitannins evaluated in Table 2. The resulting mathematical model accounted for 100% of the total dispersion, which was distributed among eight canonical functions. The two principal canonical functions 1 and 2 presented a cumulative proportion of 73.5% of total dispersion, canonical correlations of 0.962 and 0.912, and eigenvalues of 12.38 and 4.92, respectively. Figure 1 shows the graphical representation of the projections of the points of each group of wood on the plane defined by functions 1 and 2. The ellagitannins roburin C, grandinin, roburin A, and vescalagin were selected as those that provided the greatest discrimination between the nontoasted and toasted woods, as can be deduced from the standardized coefficients of discriminant functions 1 and 2, gathered in the legend of Figure 1. The graphical representation of the results of the discriminant analysis yielded a pattern of distribution of points, in which it is possible to distinguish two groups, corresponding to the nontoasted (A−G in Figure 1) and

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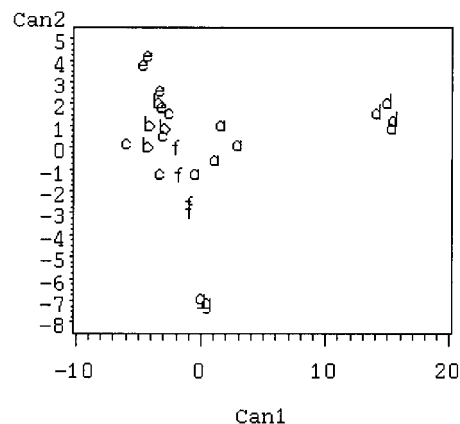*J. Agric. Food Chem.*, Vol. 49, No. 8, 2001 **3681**

Figure 1. Canonical discriminant analysis of ellagitannins of the 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*; D, *Q. faginea* (seasoned Spanish oak woods); E, *Q. robur*; F, *Q. petraea* (seasoned French oak woods); G, *Q. alba* (seasoned American oak wood); a, *Q. robur*; b, *Q. petraea*; c, *Q. pyrenaica*; d, *Q. faginea* (toasted Spanish oak woods); e, *Q. robur*; f, *Q. petraea* (toasted French oak woods); g, *Q. alba* (toasted American oak wood). Eigenvalues for discriminant functions 1 and 2 were 12.38 and 4.92, respectively. The standardized coefficients of discriminant functions 1 and 2 were, respectively, as follows: roburin A, −1.94 and −0.59; roburin B, −0.14 and −1.02; roburin C, 3.50 and 1.90; grandinin, −2.54 and 0.43; roburin D, −0.63 and −0.65; vescalagin, 1.55 and 1.61; roburin E, 0.59 and 3.11; castalagin, −0.42 and −2.56.

Figure 2. Canonical discriminant analysis of ellagitannins of the 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*; d, *Q. faginea* (Spanish oak woods); e, *Q. robur*; f, *Q. petraea* (French oak woods); g, *Q. alba* (American oak wood). Eigenvalues for discriminant functions 1 and 2 were 47.42 and 8.89, respectively. The standardized coefficients of discriminant functions 1 and 2 were, respectively, as follows: roburin A, −3.79 and −2.91; roburin B, −1.00 and −0.66; roburin C, −0.36 and −0.18; grandinin, −4.98 and −2.92; roburin D, 3.24 and 2.23; vecalagin, 0.90 and 0.75; roburin E, 6.63 and 4.44; castalagin, −1.92 and −1.33.

toasted (a−g in Figure 1) woods, with the latter showing less chemical variability among the samples, according to the dispersion of the set of points. The sets of points corresponding to the nontoasted woods (A−G) showed a pattern of distribution in which seven groups can be distinguished, related to the species and origin of woods, with overlapping between the Spanish *Q. robur* (A) and the French *Q. robur* (E) and, also, between the French *Q. petraea* (F) and the American *Q. alba* (G). However, after toasting, only American *Q. alba* (g) separated from all of the other woods (a−f in Figure 1).

Moreover, to study the variability of the toasted Spanish woods with respect to the French and American woods, we carried out another discriminant analysis, considering only the ellagitannins evaluated for the wood samples after toasting (Table 2). Figure 2 shows the graphical representation of the projection of the points of each group of wood on the plane defined by canonical functions 1 and 2. In this case, the mathematical model accounted for 100% of the total dispersion in six canonical functions, with a cumulative proportion of 95.9% in the two principal canonical functions. The sets of points of the Spanish species *Q. petraea* (b in Figure 2) and *Q. pyrenaica* (c) overlapped, now, with the French *Q. robur* (Limousin, e). The Spanish *Q. robur* (a) and *Q. faginea* (d), the French *Q. petraea* (Allier, f), and the American *Q. alba* (g) separated in groups. However, the statistical distance among the groups was marked only by the Spanish *Q. faginea* with respect to all of the other species and origins of oak woods, which was accounted for in canonical function 1. The principal discriminant variables of this function were roburin E, grandinin, roburin A, and castalagin, as can be deduced from the standardized canonical coefficients (legend of Figure 2).

DISCUSSION

Natural seasoning in the open air during 3 years caused the evolution of the tannic composition of Span-

ish oak woods. The results of the quantification of extractable ellagitannins by HPLC (Table 2) showed that they decreased during the seasoning process, especially the monomers castalagin and vescalagin. This decrease was less important during the second stage of seasoning (Table 2) than during the first (*18*). The same effect was evidenced in French oak wood (*Q. petraea*) (*21*), in which was found a regular and constant decrease of extractable polyphenols during seasoning, affecting especially monomeric and dimeric ellagitannins. These observations were explained by reactions of chemical hydrolysis or enzymatic degradation (*21*, *43*, *44*) and the oxidative polymerization of ellagitannins (*24*, *25*, *44*). Also, at a slow rate, the ellagitannins can be simply carried away by rainwater in leaching (*22*). The hydrolysis reactions must yield high concentrations of free ellagic acid, and these would explain the increase in ellagic and gallic acids (*37*) that took place simultaneously to the decrease of ellagitannins (Table 2) in the woods during seasoning. The polymerization process of ellagitannins into less soluble products would explain the lower amounts of soluble phenol (Folin−Ciocalteu index, Table 1) and the ellagitannin extracted from the 3-year-seasoned woods (Table 2) with respect to the nonseasoned ones (*15*) and, also, the increase in molecular weight (M_n and M_w) and the proportion of dimers with respect to monomers (D/M), deduced from GPC analysis (Table 3). The polyphenol polymerization during wood aging in the trunk is common and was evident in oak wood (*24*, *44*). The seasoning of oak staves could produce effects on ellagitannins similar to those described in the trunk.

The tannic composition of the Spanish oak woods evolved in the same way as that of the French and American woods during seasoning (Tables 1−3), and this evolution was in accordance with data in the literature (*21*, *24*, *44*). However, the discriminant analysis (Figure 1) permitted differentiation of Spanish woods (A−D) from French *Q. petraea* (F) and American (G) ones, after seasoning, even though, statistically, the

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chemical distance among the different species and origins was small. The French (E) and Spanish *Q. robur* (A) woods showed overlapping.

Toasting was, without doubt, the barrel cooperage process that had the greatest influence on the low molecular weight phenolic composition of wood (*17*, *27*, *28*, *37*, *45−49*); however, its effect on the tannic composition of wood was apparently not so spectacular. Toasting at medium intensity (35 min, 160−170 °C) was carried out to high reduction of levels of some ellagitannins in the surface layers of Spanish oak wood, especially the monomers (castalagin and vescalagin) and pentosylated monomers (roburin E and grandinin) (Table 2), but the response of the samples to this process was very variable and the results were of little significance. This effect is in accordance with that described in previous studies, in which a decrease in vescalagin, castalagin, and castalin contents was shown during toasting (*17*, *30*). An accentuation of this effect with increasing treatment time and temperature and even an elimination of the ellagitannins from the surface layers were also observed (*16*, *17*, *30*, *31*, *46*, *47*). In our conditions of medium toasting the ellagitannins were almost totally degraded in some oak woods such as the American one (Table 2).

Thermal degradation of the ellagitannins must go with the release of ellagic and gallic acids (*17*, *30*, *31*, *46*, *47*, *49*), even though the process does not always involve the increase of the quantities of these acids in the wood extracts, as observed in our previous studies (*37*). This is possibly due to the fact that ellagic acid is so poorly extracted by maceration that its content sometimes seems not to increase and that gallic acid is so extremely sensitive to heat treatment that its content systematically quickly decreases in the wood with the duration of the toasting (*47*, *49*).

Referring to the results obtained on optical density, Folin−Ciocalteu index, and ellagitannin total content (Table 1), our results were, basically, in line with those published in other papers (*30*, *46*, *47*). The darkening of the toasted wood extracts with respect to the non-toasted ones, measured by optical density at 420 nm (Table 1), denotes changes in the oxidation degree of these extracts, but it is not an indicator of the amount of phenolic breakdown because this wavelength is strongly influenced by the Maillard reaction products induced by the heat in the wood (*47*). On the other hand, the Folin−Ciocalteu index (Table 1) did not undergo any decrease, possibly due to the fact that it is not specific for the quantification of tannins. It also includes low molecular weight phenols, which showed high increases with toasting (*37*), and also the significant synthesis of furanic aldehydes strongly affects the specificity of the reaction with phenolic compounds in the case of toasted oak woods (*47*). Consequently, these parameters must be taken into account only as values used to compare the Spanish species with the French and American ones and not for phenolic measurements. Thermal degradation of the ellagitannins, shown by HPLC analysis (Table 2), is, however, revealed by the decreasing total content of ellagitannin, obtained by the analysis of the ellagic acid yielded after they were submitted to methanolysis (Table 1).

Moreover, the greater effect that the toasting stage had on the monomeric ellagitannins (Table 2) explains the changes observed in the molecular weight distribution curves (Table 3), which showed a tendency to increase the value of M_n and D/M. Both the dimerization and hydrolysis reactions of ellagitannins could have influenced these changes (*24*). Referring to the dimerization reaction, it was proved that vescalagin is a common precursor of all dimers, which are probably formed by a nonenzymatic reaction (*44*).

It was clear that toasting in barrel cooperage has a considerable influence on the tannic composition of wood. It was observed that the quality and quantity of phenolic compounds are closely related to toasting intensity and its conditions (*17*, *30*, *46*, *47*), but the particular characteristics of each species could determine the rate of modification in toasted wood, as has been shown in previous studies on the low molecular weight phenolic compounds of the same woods (*37*). The ellagitannins of Spanish oaks varied in the same way as the French and American ones after having been treated under the same toasting conditions. Their natural variability among species and origins decreased after toasting, which led to woods with similar ellagitannic compositions (Tables 2 and 3 and Figure 1). The Spanish woods also showed similar values of optical density and Folin−Ciocalteu index (Table 1), although these cannot be taken into account as a phenolic measurement. These results, and the fact that only a low proportion of the total ellagitannins is extracted from the wood into the wine (*50*), are important when the significance of the natural variability of the oak woods is evaluated in the context of other sources of variation such as the treatment of the wood during coopering and other wine-making factors.

If we also consider the studies carried out to date on the low molecular polyphenolic composition (*32*, *33*, *37*) and on the tannic composition of Spanish oak wood (*15*, *35*), in relation to French and American ones, we can conclude that Spanish oak wood can be considered to be suitable for barrel production according to its phenolic compounds, because the natural polyphenolic variability among these species and origins is based only on quantitative differences, which often decrease with the seasoning and toasting processes.

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