JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 9631–9640 9631
DOI:10.1021/jf102718t

Phenolic Compounds in Chestnut (*Castanea sativa* Mill.) Heartwood. Effect of Toasting at Cooperage

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The phenolic and tannic composition of heartwood extracts from *Castanea sativa* Mill., before and after toasting in cooperage, were studied using HPLC-DAD and HPLC-DAD/ESI-MS, and some low molecular weight phenolic compounds and hydrolyzable tannins were found. The low molecular weight phenolic compounds were lignin constituents as the acids gallic, protocatechuic, vanillic, syringic, ferulic, and ellagic, the aldehydes protocatechuic, vanillic, syringic, coniferylic, and sinapic, and the coumarin scopoletin. Their patterns were somewhat different those of oak because oak does not contain compounds such protocatechuic acid and aldehyde and is composed of much lower amounts of gallic acid than chestnut. Vescalagin and castalagin were the main ellagitannins, and acutissimin was tentatively identified for the first time in this wood. Moreover, some gallotannins were tentatively identified, including different isomers of di, tri, tetra, and pentagalloyl glucopyranose, and di and trigalloyl-hexahydroxydiphenoyl glucopyranose, comprising 20 different compounds, as well as some ellagic derivatives such as ellagic acid deoxyhexose, ellagic acid dimer dehydrated, and valoneic acid dilactone. These ellagic derivatives as well as some galloyl and hexahydroxydiphenoyl derivatives were tentatively identified for the first time in this wood. The profile of tannins was therefore different from that of oak wood because oak only contains tannins of the ellagitannins type. Seasoned and toasted chestnut wood showed a very different balance between lignin derivatives and tannins because toasting resulted in the degradation of tannins and the formation of low molecular weight phenolic compounds from lignin degradation. Moreover, the different toasting levels provoked different balances between tannins and lignin constituents because the intensity of lignin and tannin degradation was in relation to the intensity of toasting.

KEYWORDS: *Castanea sativa*; chestnut; heartwood; tannins; phenolic compounds; toasting

INTRODUCTION

The cooperage industry wants to offer the highest possible variety of wood products that can be used in the production of wine, spirits, and other beverages and even certain sauces. Thus, the wood is used in many ways: for manufacturing containers from large vats to barrels and, in recent years, for pieces of many sizes (powder, shavings, chips, cubes, and staves) used in cheaper alternative techniques. Usually oak (*Quercus* spp) pieces are used, but other woods may be considered in order to give a particular personality to the products. Thus, species like chestnut (*Castanea sativa*), cherry (*Prunus avium*), acacia (*Robinia pseudoacacia*) and, more rarely, ash (*Fraxinus excelsior* and *Fraxinus americana*) and mulberry (*Morus alba* and *Morus nigra*) have been considered as possible sources of wood in cooperage (*1*−*3*). During the interaction between woods and beverages, the latter undergoes a series of processes that cause important changes in aroma, color, taste, and astringency because of the extraction of certain compounds present in the wood which are transferred to the beverages, as well as the permeation of oxygen through barrel staves, due to wood porosity. Therefore, the chemical composition and physical−mechanical properties of the wood used in manufacturing containers has a great influence on the characteristics of aged beverages and sauces.

The chemical composition of oak wood, the main wood in cooperage, has been broadly studied. Oak heartwood shows high levels of ellagitannins, hydroxybenzoic and hydroxycinnamic acids and aldehydes, and volatile compounds that can vary greatly depending on the species and geographical origin of the wood as well as the processing it undergoes in cooperage. The most abundant polyphenols are the monomer ellagitannins, castalagin, roburin E, vescalagin, and grandinin, and low molecular weight phenolic compounds such as ellagic and gallic acids, besides lignin constituents, especially vanillin. It also provides a

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Published on Web 08/05/2010

lot of volatile compounds which contribute to the aroma and flavor of aged wines, the *cis* and *trans* isomers of β-methyl-γ-octalactone being the most characteristic (*4*−*6*).

Chestnut barrels were widely used in Mediterranean areas in the past because it was cheap and easy to find. This wood is characterized by lower resistance to liquid and gas diffusion and higher total content of low molecular weight polyphenols than oak (*7*, *8*). Because of its richness in gallic acid and hydrolyzable tannins, chestnut commercial tannin agents are used as enological tannins (*9*−*11*). During seasoning and toasting in cooperage, a similar evolution to oak wood of some low molecular weight phenolic and volatile compounds was found (*1*, *12*). Using GC-MS to analyze 50% hydroalcoholic and model wine extracts, many volatile compounds were identified in seasoned (*13*) and toasted chestnut heartwood, including 92 lignin, carbohydrate, and lipid derivatives, with this wood being the richest on the whole, when compared to acacia, cherry, ash, and oak heartwood (*8*). Studies carried out on its use in the aging of wines and spirits, focused on some low molecular weight polyphenols and volatile compounds (*14*, *15*), point out the remarkable quality of brandies aged in chestnut wood. However, a more recent study on the evolution of myricetin glucoside, quercetin glucoside, (+)-catechin, and monoglucosides and acylated anthocyanidins in an Italian wine aged in a chestnut barrel (*3*), did not find prominent differences when comparing this wood with other species, like acacia, cherry, mulberry, or oak. On the other hand, sherry and balsamic vinegars during acetification in chestnut wood showed a substantial increase of gallic acid and gallic ethyl ester, as well as the total polyphenols index (*2*), related to chestnut polyphenol composition.

The objective of this work is to study the polyphenolic composition, mainly the tannic one, of chestnut (*Castanea sativa*) heartwood and its possible changes during the toasting process, with the purpose of completing its chemical characterization with an eye toward its use in cooperage, and to find out what effects it may have on the sensory characteristics of the wines, vinegars, and other drinks aged in this wood, always taking oak wood as reference.

MATERIALS AND METHODS

Wood Samples. Chestnut (*Castanea sativa*) heartwood from France was provided as staves for making barrels by Tonelería Intona, SL (Navarra, Spain). The wood was naturally seasoned for 24 months and toasted at two intensities, 165 °C for 35 min and 185 °C for 45 min, in an industrial kiln specially designed for toasting staves. Samples were taken before and after toasting, 10 staves of each. Several wood pieces were cut out of each stave, and the pieces were ground, sieved, and mixed, taking the sawdust ranging from 0.80 to 0.28 mm of size. The number of staves was chosen in that way because our objective was to study the general phenolic profile of this wood both before and after toasting, without going deeply into their natural variation.

Chemicals. Reference compounds were obtained from commercial sources: gallic acid, methyl gallate, and protocatechualdehyde (Fluka Chimie AG, Buchs, Switzerland), protocatechuic acid, syringaldehyde, scopoletin, and coniferyl aldehyde (Aldrich Chimie, Neu-Ulm, Germany), ellagic acid (Apin, Oxon, U.K.), (+)-catechin, vanillin, and syringic acid (Sigma Chemical, St. Louis, MO), and vanillic acid, and sinapaldehyde (Extrasynthèse, Genay, France). Standards of vescalagin, castalagin, roburins A and E, and grandinin were kindly provided by Dr. Scalbert. Methanol, diethyl ether, ethyl acetate, anhydrous sodium sulfate, and phosphoric acid were purchased from Panreac (Barcelona, Spain). Acetic acid and methanol HPLC grade were from Scharlab (Barcelona, Spain).

Extraction of Phenolic Compounds. The sawdust (1 g) was extracted with 100 mL of methanol/water (1:1) at room temperature (20 ± 2 °C) and in darkness for 24 h. The extracts were filtered in a Büchner funnel, and the methanol was removed in a rotary evaporator at a temperature below 40 °C. An aliquot part (3 mL) of this aqueous solution (solution I) was used for a global valuation of total polyphenols. The remainder solution I was extracted three times with 20 mL of diethyl ether and after that also three times with 20 mL of ethyl acetate. The remaining aqueous solution was freeze-dried. The two organic fractions were dried with 20 g of anhydrous sodium sulfate, evaporated in a rotary evaporator at a temperature below 40 °C, and the residuum redissolved in 1 mL of methanol/water 50%. These extracts and an aliquot part of freeze-dried extract redissolved in water (30 mg/mL), were used for the HPLC-DAD and LC-DAD/ESI-MS analyses described below. Moreover, the ethyl acetate and freeze-dried extracts were used for the global quantification of tannins. In diethyl ether extract, tannins were not detected. All the extractions were carried out in duplicate.

Global Valuations. In solution I, total polyphenols were determined by the Folin−Ciocalteu assay with gallic acid as standard (*16*). In ethyl acetate and freeze-dried extract were determined condensed tannins by the vanillin method with (+)-catechin as standard (*17*), and hydrolyzable tannins by HPLC quantification of gallic and ellagic acid released after acid methanolysis (*18*). All determinations were carried out in duplicate.

HPLC/DAD Analysis. The analyses were carried out using an Agilent 1100 L liquid chromatography system equipped with a diode array detector (DAD) and managed by a Chemstation for LC 3D systems Rev B.03.02 (Agilent Technologies, Palo Alto, CA,). The column was a 200 mm × 4 mm i.d., 5 μm, Hypersil ODS C18, maintained at 30 °C and protected with a 4 mm × 4 mm i.d. guard column of the same material (Agilent Technologies). The HPLC profiles were monitored at 255 ± 2, 280 ± 2, 325 ± 75, and 340 ± 15 nm, and the UV/vis spectra were recorded from 190 to 650 nm. The volume injected was 20 μL. With the diethyl ether and ethyl acetate extracts the elution method involved a multistep linear solvent gradient changing from a starting concentration of 100% phosphoric acid (0.1%) (eluent A) going to 85% (20 min), 75% (30 min), 50% (50 min), and 0% (70 min), using methanol/phosphoric acid 0.1% as eluent B. The total time of analysis was 70 min, equilibration time 10 min, and flow rate 1 mL/min. With the same eluents, the elution gradient to analyze the freeze-dried extract was: from 100% of A to 95% in 50 min, going to 70% (85 min.), and 0% (105 min.), with 10 min as equilibration time. Quantification was carried out by the external standard method, using peak areas in UV at 325 ± 75 nm. The concentration of each substance was measured by comparing it with calibrations made with the pure compound analyzed under the same conditions and linear regression coefficients between 0.9990 and 0.9999 were obtained. In general, more than one linear regression was made for each compound, at different concentration levels. Gallic and hexahydroxydiphenoyl derivatives were quantified as gallic acid, and ellagic derivatives as ellagic acid, in agreement with their UV profile. Vescalin and acutissimin were quantified as vescalagin, castalin as castalagin, and roburin D as roburin A. The samples were analyzed in duplicate.

LC-DAD/ESI-MS Analysis. Chromatographic separations were performed on an Agilent series 1100 (Palo Alto, CA) chromatography system equipped with a diode array detector and a quadrupole mass spectrometer (Agilent series 1100 MSD) with an electrospray interface. The binary mobile phase consisted of solvents A (2% acetic acid in HPLC grade water) and B (HPLC grade methanol). The column, gradient, the volume injection, and the temperature of the analytical column were the same as that referred to above for the HPLC analysis. The flow rate was fixed at 0.7 mL/min during the entire chromatographic process. The DAD was set at 255, 280, 325, and 340 nm to monitor the UV/vis absorption. The UV/vis spectra were recorded from 190 to 650 nm. ESI parameters were as follows: drying gas (N_2) flow, 10 L/min, temperature, 350 °C, a nebulizer pressure 55 psi (380 Pa), and capillary voltage, 4000 V. Mass spectra were acquired using electrospray ionization in the negative mode at the voltage gradient: m/z 0−200, 80 V fragmentation voltage, m/z 200−3000, 200 V fragmentation voltage, and recorded for the range of m/z 100−3000.

RESULTS AND DISCUSSION

Identification of Phenolic Compounds. Figure 1 illustrates the HPLC-DAD chromatograms of the phenolic compounds from seasoned and medium toasted chestnut heartwood in diethyl ether and ethyl acetate extracts. As can be seen, the four chromatograms show qualitative and especially quantitative

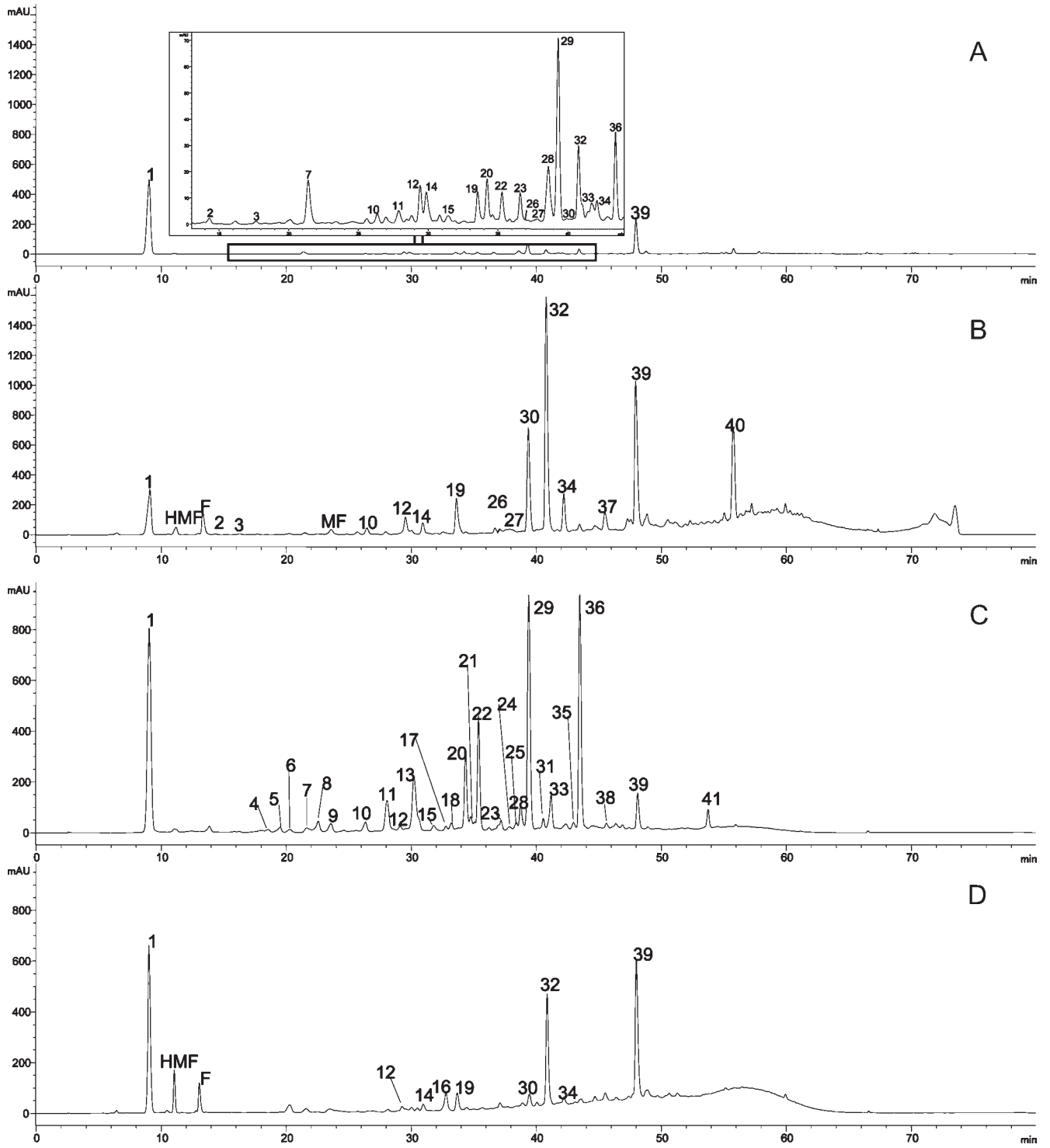

Figure 1. HPLC-DAD chromatograms of *Castanea sativa* heartwood extracts, monitored at 325 ± 75 nm. (**A**) Diethyl ether extract of seasoned heartwood. (**B**) Diethyl ether extract of toasted heartwood. (**C**) Ethyl acetate extract of seasoned heartwood. (**D**) Ethyl acetate extract of toasted heartwood. Peak numbers shown in **Tables 1** and **2**.

differences related to both the different efficiency of extraction of phenolic compounds by solvents used, especially for seasoned wood, and the condition of the wood (seasoned or toasted). Thus, we have separated almost all low molecular weight phenolic compounds in diethyl ether extract, and gallic and ellagic derivatives in ethyl acetate extract, being gallic and ellagic acid in similar amounts in the two extracts, and remaining ellagitannins in the aqueous solution as we can expect taking into account data in literature (*5*, *19*). The peaks have been numbered in agreement with retention times, using the same number in the different chromatograms for the same compound. Among them, 13 phenolic compounds have been identified by comparing their retention time and UV and mass spectra with those of the standards. In addition, 23 peaks corresponding to compounds with related structures were tentatively identified as hydrolyzable tannins on the basis of their retention times, UV spectra, and MS pattern and also taking into account data in the related literature. Most of them were gallic and ellagic acid derivatives with a cyclic glucose core, in the form of either galloyl esters of glucopyranose or a combination of galloyl and hexahydroxydiphenoyl esters of

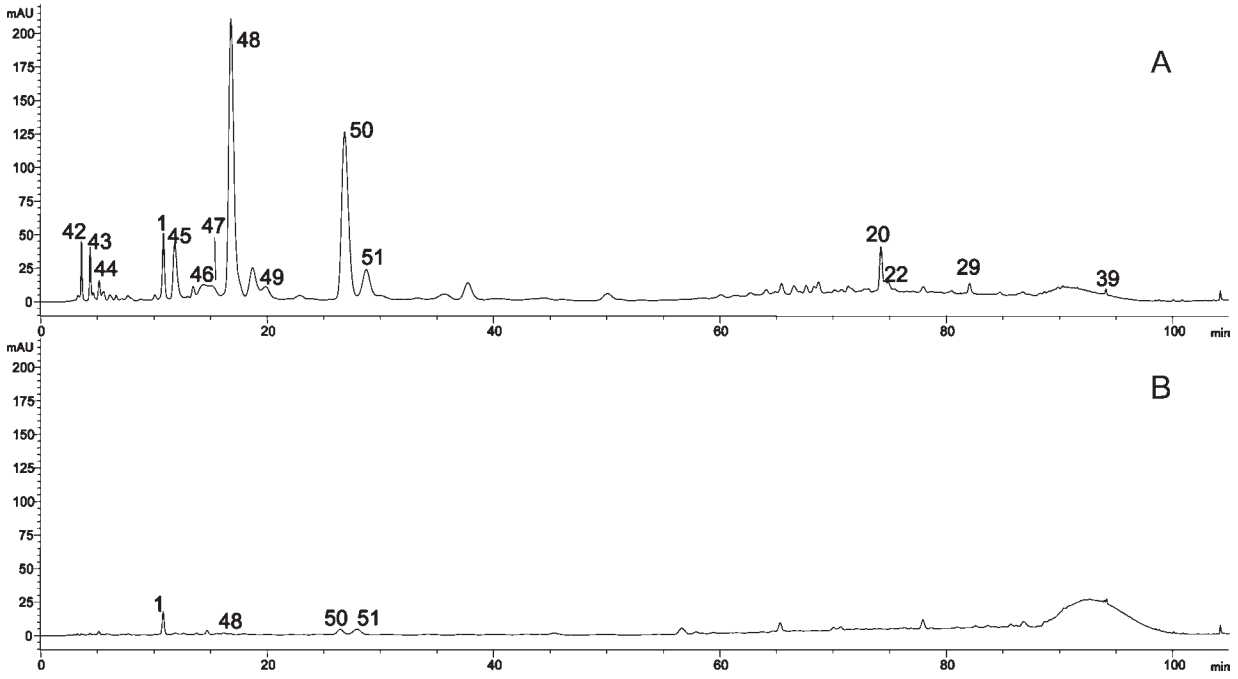

Figure 2. HPLC-DAD chromatograms of *Castanea sativa* heartwood extracts, monitored at 325 ± 75 nm. (**A**) Freeze-dried aqueous fraction of seasoned heartwood extract. (**B**) Freeze-dried aqueous fraction of toasted heartwood extract. Peak numbers shown in **Tables 1**−**3**.

Table 1. Spectroscopic and Spectrometric Data of Low Molecular Weight Phenolic Compounds in Seasoned and Toasted *Castanea sativa* Heartwood[a]

peak	R_t	compd	λ_{max}	$[M-H]^-$	negative ions *m*/*z* (% in MS) [attribution]
1	9.0	gallic acid	272	169	169 (100) $[M-H]^-$; 125 (25) $[M-H-CO_2]^-$
2	14.3	protocatechuic acid	297, 258	153	153 (100) $[M-H]^-$; 109 (43) $[M-H-CO_2]^-$
10	26.4	vanillic acid	260, 292	167	167 (100) $[M-H]^-$; 152 (34) $[M-H-CH_3]^-$
14	30.9	syringic acid	274	197	197 (100) $[M-H]^-$; 153 (23) $[M-H-CO_2]^-$
27	38.3	ferulic acid	238, 290sh*, 322	193	193 (100) $[M-H]^-$; 178 (25) $[M-H-CH_3]^-$
39	47.1	ellagic acid	254, 368	301	301 (100) $[M-H]^-$
3	17.6	protocatechualdehyde	280, 310	137	137 (100) $[M-H]^-$
12	29.4	vanillin	280, 312	151	151 (100) $[M-H]^-$; 136 (86) $[M-H-CH_3]^-$
19	33.6	syringaldehyde	232sh, 308	181	181 (100) $[M-H]^-$; 166 (61) $[M-H-CH_3]^-$; 151 (31) $[M-H-CH_3]^-$
30	39.3	coniferaldehyde	290sh, 322	177	177 (100) $[M-H]^-$; 162 (41) $[M-H-CH_3]^-$
32	40.8	sinapaldehyde	300sh, 338	207	207 (36) $[M-H]^-$; 192 (100) $[M-H-CH_3]^-$
26	37.6	scopoletin	258sa, 294sh, 342	191	191 (55) $[M-H]^-$; 176 (100) $[M-H-CH_3]^-$
40	55.8	unknown	302sh, 332		479 (50); 329 (70); 313 (90); 301 (90); 299 (100)

[a] Rt expressed in min; λmax in nm; [M− H]− in m/z, * = shoulder.

glucopyranose. Moreover, we show in **Figure 2** the HPLC-DAD chromatograms of freeze-dried aqueous fractions of the extracts. In these fractions, another nine hydrolyzable tannins were tentatively identified including some based on an acyclic glucose core and a nonahydroxytriphenoyl group. Along with these compounds, chestnut heartwood extracts showed another six peaks, all for which LC-MS and UV data were obtained, which remain, as yet, unknown compounds. Relevant information concerning the identified compounds obtained from DAD and ESI-MS is shown in **Tables 1**−**3**: λ_{max} as well as shoulders if they exist from UV spectra, fragment ions observed in negative ionization mode, their percentage in the MS, and the structure attribution of ions.

Some low molecular weight phenolic compounds were identified (**Table 1**). Thus, we found the acids gallic (peak 1), protocatechuic (2), vanillic (10), syringic (14), ferulic (27), and ellagic (39), the aldehydes protocatechuic (3), vanillic (12), syringic (19), coniferylic (30), and sinapic (32), and the coumarin scopoletin (26). Except in the last two, the respective $[M-H]^-$ deprotonated molecule was the base peak in the MS pattern. Gallic, protocatechuic, and syringic acids also gave $[M-H-44]^-$ fragment ion via loss of a CO_2 group from the carboxylic acid moiety. The fragmentation of methoxylated acids (vanillic and ferulic) and methoxylated aldehydes (vanillin, syringaldehyde, and coniferaldehyde) produced, besides the deprotonated molecule, an anion radical by losing a methyl group, with *m*/*z* ($[M-H-15]^-$). Syringaldehyde mass spectrum also shows a loss of another methyl group. The mass spectrum of the more retained hydroxycinnamic aldehyde, the sinapaldehyde, and of the coumarin scopoletin also gave the deprotonated molecule $[M-H]^-$ at *m*/*z* 207 and 191, respectively, although the main fragment present is due to the loss of a methyl group. All these identities were confirmed with the authentic standards. Unfortunately, it was not possible to identify peak 40 that was only detected in toasted wood, showing an UV spectrum quite similar to those of hydroxycinnamic aldehydes. Its MS gave the ion at *m*/*z* 301, characteristic of ellagic acid, but no attribution was feasible to other ions. In the literature, these compounds were found in unseasoned and seasoned chestnut heartwood by Canas et al. (*7*, *12*, *14*), except protocatechuic acid and aldehyde, so that as far as we know these have now been identified for the first time in chestnut wood. They also found the coumarin umbeliferone, but using a fluorescence detector and at concentrations lower than 0.01 μg/g of dry wood (*12*).

Table 2. Spectroscopic and Spectrometric Data of Hydrolyzable Tannins with UV Spectrum like Gallic or Ellagic Acid, in Seasoned and Toasted *Castanea sativa* Heartwood[a]

peak	R_t	compd	λ_{max}	$[M-H]^-$	negative ions *m/z* (% in MS) [attribution]
			Galloyl and Hexahydroxydiphenoyl Derivatives		
7	21.6	methyl gallate	274	183	183 (100) $[M-H]^-$
8	22.6	digalloyl glucose	274	483	483 (100) $[M-H]^-$; 313 (70) [M − H − gallic acid]$^-$; 271 (37); 211 (93); 169 (35) [gallic acid − H]$^-$
4	18.5	digalloyl-HHDP-glucose	272	785	785 (100) $[M-H]^-$; 633 (5) [M − H − galloyl]$^-$; 615 (10) [M − H−gallic acid]$^-$; 483 (35) [M − H − HHDP]$^-$; 419 (15); 301 (83) [M − H − digalloyl glucose]$^-$
5	19.5	digalloyl-HHDP-glucose[b]	276	785	785 (100); 633 (8); 615 (10); 483 (27); 419 (12); 301 (83)
13	30.2	digalloyl-HHDP-glucose[b]	276	785	785 (100); 633 (6); 483 (35); 419 (10); 301 (60)
17	33.2	digalloyl-HHDP-glucose[b]	276	785	785 (75); 633(20); 483 (23); 419 (8); 301 (100)
11	28.1	trigalloyl glucose	276	635	635 (74) $[M-H]^-$; 483 (100) [M − H − galloyl]$^-$; 465 (75) [M − H − gallic acid]$^-$; 313 (65) [M − H − galloyl − gallic acid]$^-$; 271 (27); 211 (41); 169 (10) [gallic acid − H]$^-$
15	31.8	trigalloyl glucose[c]	276	635	635 (80)$[M-H]^-$; 483 (100) [M − H − galloyl]$^-$; 465 (85) [M − H − gallic acid]$^-$; 423 (12); 313 (80) [M −H − galloyl − gallic acid]$^-$; 271 (17); 211 (41); 169 (12) [gallic acid − H]$^-$;
18	33.8	trigalloyl glucose[c]	276	635	635 (100); 483 (25); 465 (70); 423 (15); 313 (45); 271 (10)
20	34.3	trigalloyl glucose[c]	276	635	635 (100); 483 (22); 465 (70); 313 (53); 211 (25); 169 (49)
22	35.4	trigalloyl glucose[c]	278	635	635 (100); 483 (10); 465 (95); 313 (67); 211 (25); 169 (8)
25	38.4	trigalloyl-HHDP-glucose	278	937	937 (100) $[M-H]^-$; 767 (20) [M− H − gallic acid]$^-$; 635 (42) [M − H − HHDP]$^-$; 465 (95) [M − H − gallic acid − HHDP]$^-$; 301 (75) [ellagic acid − H]$^-$
21	34.7	tetragalloyl glucose	275	787	787 (75) $[M-H]^-$; 635 (95) [M − H − galloyl]$^-$; 617 (10) [M − H − gallic acid]$^-$; 447 (8) [M − H − galloyl − H_2O]$^-$; 465 (100) [M − H − gallic acid − galloyl]$^-$; 313 (80) [M − H − gallic acid − digalloyl]$^-$; 169 (10) [gallic acid − H]$^-$
23	37.2	tetragalloyl glucose[d]	272	787	787 (30); 635 (90); 617 (15); 465 (100); 313 (65)
24	37.8	tetragalloyl glucose[d]	272	787	787 (100); 635 (56); 617 (40); 465 (100); 313 (25)
29	39.2	tetragalloyl glucose[d]	278	787	787 (100); 635 (15); 617 (70); 465 (57); 313 (10); 169 (10)
31	40.6	tetragalloyl glucose[d]	276	787	787 (100); 635 (45); 617 (30); 465 (70); 313 (15) ;
33	41.2	tetragalloyl glucose[d]	278	787	787 (40); 617 (100); 465 (30); 447 (8); 313 (12); 169 (5)
35	43.0	tetragalloyl glucose[d]	276	787	787 (84); 635 (47); 617 (77); 483 (41); 465 (100); 313 (35)
36	43.5	pentagalloyl glucose	280	939	939 (100) $[M-H]^-$; 787(25) [M − H − galloyl]$^-$; 769 (40) [M − H− gallic acid]$^-$; 617 (18) [M − H − galloyl − gallic acid]$^-$; 465 (15) [M − H − 2 × galloyl−gallic acid]$^-$
38	45.6	galloyl-valoneic acid bilactone	278	621	621 (100) $[M-H]^-$; 469 (40) [M − H − galloyl]$^-$; 301 (100) [ellagic acid − H]$^-$
6	18.0	unknown	282		719 (90); 347 (100); 258 (45)
37	45.5	unknown	274		361 (100)
			Ellagic Derivatives		
9	23.6	ellagic acid deoxyhexose	250, 372	447	447(100) $[M-H]^-$; 301 (70) [M − H − deoxyhexose]$^-$
28	38.8	valoneic acid dilactone	256, 364	469	469 (90) $[M-H]^-$; 425 (75) [M − H − CO_2]$^-$; 301 (100) [ellagic acid − H]$^-$
41	53.7	ellagic acid dimer dehydrated	254, 361	585	585 (62) $[M-H]^-$; 415 (19); 301 (100) [ellagic acid − H]$^-$
16	32.8	unknown	252, 360		493 (48); 301 (100) [ellagic acid − H]$^-$
34	42.2	unknown	252, 360		687 (100); 331 (90); 287 (50); 259 (58); 203 (100)

[a] R_t expressed in min; λ_{max} in nm; $[M-H]^-$ in m/z; HHDP = hexahydroxydiphenoyl. [b] Attribution of ions of mass spectrum as in peak 4. [c] Attribution of ions of mass spectrum as in peak 11. [d] Attribution of ions of mass spectrum as in peak 21.

The UV spectra of most of the remaining peaks found in these extracts (**Table 2**) showed that they could be arranged into two group, those showing a characteristic UV spectrum of ellagic acid (254 nm, 358 nm) and those showing UV spectra with a single maximum similar to that of gallic acid (272 nm). According to Cantos et al. (*20*), the first group includes all the compounds that present an ellagic residue in their molecular structure, and the second group includes all of the galloyl and hexahydroxydiphenoyl (HHDP) derivatives. The most frequents were peaks with gallic acid-like UV spectrum with a single maximum at 274−280 nm. All of them were tentatively identified as gallic acid derivatives, in the form of galloyl esters of glucopyranose or the combination of galloyl and hexahydroxydiphenoyl esters of glucopyranose (*20*), as well as the methyl ester of gallic acid. In accordance with the mass spectra data in **Table 2**, these compounds were designated as different isomers of di, tri, tetra, and pentagalloyl glucopyranose and di and trigalloyl-hexahydroxydiphenoyl glucopyranose.

According to the literature, the main characteristic of these compounds was the deprotonated molecule $[M-H]^-$ (*m/z* 483, 635, 787, 939, 785, 937, respectively) and the loss of one or more galloyl groups (152 mass units) and/or gallic acid (170 mass units) (*20*−*22*). Compound **8** gave a $[M-H]^-$ ion at *m/z* 483 and several other peaks at *m/z* 313, 271, 211, and 169, the first one attributed to the loss of gallic acid and the others common to galloyl glucose fragmentation. This MS spectrum revealed a typical fragmentation pattern of digalloyl glucose, and it is in agreement with those reported in the literature for this compound (*20*, *23*, *24*).

Trigalloyl glucose structure (peaks 11, 15, 18, 20, and 22) provided $[M-H]^-$ ions at *m/z* 635 and peaks of *m/z* 483, 465, and 313, caused by the loss of galloyl residue, gallic acid, and both, respectively, as well as other fragments that are common to digalloyl glucose (*m/z* 271, 211, and 169) (*20*, *23*, *24*). Compounds **21**, **23**, **24**, **29**, **31**, **33**, and **35** gave a $[M-H]^-$ ion at *m/z* 787 and

Table 3. Spectroscopic and Spectrometric Data of Hydrolyzable Tannins Found only in Freeze-Dried Fraction of Extracts of Seasoned and Toasted *Castanea sativa* Heartwood[a]

peak	R_t	compd	λ_{max}	$[M-H]^-$	negative ions *m/z* (% in MS) [attribution]
42	3.6	vescalin	229, 280sh*	631	631 (100) $[M-H]^-$; 613 (35)) $[M-H-H_2O]^-$
43	4.4	castalin	229, 280sh	631	631 (100) $[M-H]^-$
48	16.8	vescalagin	229, 280sh	933	933 (100) $[M-H]^-$; 915 (52) $[M-H-H_2O]^-$; 631 (7) [M − H −ellagic acid]$^-$; 613 (8) [M − H − ellagic acid−H_2O]$^-$; 301 (37) [ellagic acid−H]$^-$
50	26.8	castalagin	229, 280sh	933	933 (100) $[M-H]^-$; 631 (25) [M − H − ellagic acid]$^-$; 301 (35) [ellagic acid− H]$^-$
49	19.9	roburin E	229, 280sh	1065	1065 (30) [M− H]$^-$; 915 (30) [vescalagin − H_2O−H]$^-$; 301 (100) [ellagic acid − H]$^-$
46	14.4	grandinin	229, 280sh	1065	1065 (35) $[M-H]^-$; 602 (30); 493 (50); 301 (100) [ellagic acid −H]$^-$
45	11.8	roburin A	229, 280sh	1849	1849 (5) $[M-H]^-$; 933 (15) [vescalagin − H]$^-$; 924(15) $[M-2H]^{2-}$; 915 (30) [vescalagin − H_2O − H]$^-$; 616 (100) $[M-3H]^{3-}$; 301(100) [ellagic acid − H]$^-$
47	15.1	roburin D	229, 280sh	1849	933 (15) [vescalagin−H]$^-$; 924 (15) $[M-2H]^{2-}$; 915 (35) [vescalagin − H_2O−H]$^-$; 616 (50) $[M-3H]^{3-}$; 301 (100) [ellagic acid − H]$^-$
51	28.8	acutissimin A	229, 280sh	1205	1205(10) $[M-H]^-$; 915 (30); 613 (30); 301 (100)
44	5.2	unknown	229, 280sh		1209 (15); 933 (10); 625 (20); 481 (90); 301 (100); 275 (40); 257 (17)

[a] *R*t expressed in min; λ_{max} in nm; $[M-H]^-$ in m/z; * = shoulder.

were assigned to tetragalloyl glucopyranose structures. Their fragmentation pattern involved the loss of galloyl residue or gallic acid from the $[M-H]^-$ ion (*m/z* 635 and 617). Further loss of galloyl and gallic acid moieties was also observed (*m/z* 465 and 313), and the MS spectrum revealed that in some cases these peaks are the most prominent ones (compounds **21**, **23**, **24**, and **35**). The same fragmentation pattern was observed for compound **36**, which provided a base peak of *m/z* 939. Other major fragments for this peak were observed at *m/z* 787, 769, 617, and 465 and attributed to galloyl/gallic acid moieties and the sequential loss of galloyl residues. This fragmentation pattern led to the assignment of compound **36** as pentagalloyl glucose.

Compounds **4**, **5**, **13**, and **17** with an *m/z* of 785 were identified as isomers of digalloyl-HHDP-glucopyranose. The $[M-H]^-$ ion suffered the loss of galloyl and hexahydroxydiphenoyl moieties (*m/z* 633 and 483, respectively). The fragment at *m/z* 301 besides the [M −302− H]$^-$ ion showed evidence of the presence of an HHDP group [ellagic − H]$^-$ in the molecule (*25*) while the fragment at *m/z* 169 indicates the presence of free galloyl residues. These trigalloyl-HHDP-glucose isomers had an additional fragment at *m/z* 419 resulting from the loss of a gallic acid and galloyl moiety and the descarboxylation of a gallic acid group. This fragment indicates that either one or both gallic acid groups are ether linked through a hydroxyl group on the gallic acid and not esterified to the glucose core (*26*). Trigalloyl-HHDP-glucose (compound **25**) showed several peaks including the base peak ion at *m/z* 937 and fragments at *m/z* 767, 635, 465, and 301. The fragment at *m/z* 767 was attributed to the loss of gallic acid from the glucosyl unit and the *m/z* 635 and 465 fragments were due to the loss of HHDP moieties from the $[M-H]^-$ and [M − H − gallic acid]$^-$ ions respectively. Again, evidence of an HHDP group was observed (*m/z* at 301 and $[M-302-H]^-$ ion) as one of the major fragments in the mass spectrum.

Peaks 7 and 38 also showed gallic acid-like UV spectra. The first, showing a $[M-H]^-$ ion at *m/z* at 183, was identified as methyl gallate by comparing both UV and MS spectra with those of the available commercial standard. Peak 38 presented a fragmentation pattern (*m/z* 469, 425, 301) well-matched to the structure of valoneic acid dilactone (*23*), and a deprotonated molecule at *m/z* 621, probably caused by the loss of a galloyl moiety. It was tentatively identified as galloyl-valoneic acid dilactone.

Peaks 9, 28, and 41 showed the characteristic UV spectrum of ellagic acid. Among them a dehydrated ellagic acid dimer (41) was tentatively identified. Its MS data (*m/z* 585, 415, and 301) were in agreement with those previously reported for this compound (*20*). Peak 28 was also tentatively identified as valoneic acid dilactone. It provided a $[M-H]^-$ ion at *m/z* 469 and two main fragments at *m/z* 425 and 301 due to the loss of CO_2 from the deprotonated molecule and to the ellagic acid fragment (*20*, *23*, *26*). Another peak (9) was tentatively identified as an ellagic acid deoxyhexose conjugate, according to the literature data (*23*). The MS spectrum of this compound yielded a molecular ion at *m/z* 447 and an intense ion at *m/z* 301 [ellagic acid]$^-$ caused by the loss of a deoxyl hexose unit.

Last, four peaks in **Figure 1** remain unidentified. Peak 6 shows UV spectrum like gallic acid, but it was not possible to attribute the *m/z* of the main ions of its MS (719, 347, and 258) to known fragments of gallo- or ellagitannins. The same thing happened with peaks 16, 34, and 37, which were only detected in toasted wood. Peak 16 shows a UV spectrum like ellagic acid. Its MS provided an ion at *m/z* 493, in agreement with the quasimolecular ion of monogalloyl diglucose (*27*). However, it also provided an intense ion at *m/z* 301 [ellagic acid]$^-$, in discrepancy with this structure. Peak 34 shows the same kind of UV spectrum. In its MS, two of the ions (331 and 287) are in agreement with the structure of monogalloyl glucose structure (*23*), but no attribution was possible to the other intense ions (*m/z* 687 and 203).

Using LC-ESI-MS/MS (*21*), or MALDI-TOF-MS (*28*, *29*), some of these compounds (mono, di, tri, and pentagalloyl glucose) were found in chestnut-derived commercial tannin agents, which are obtained by water extraction. This commercial tannins extracts are mainly composed of long galloyl glucose chains of mixed units, in some cases up to 16 or 17 units long. However, as far as we know, this is the first time these compounds have been identified in chestnut heartwood, especially tetragalloyl glucose, those including HHDP units, and ellagic acid derivatives.

On the other hand, nine hydrolyzable tannins were only found in the freeze-dried aqueous fraction of these extracts (**Figure 2**). In **Table 3**, we can see that their UV spectra have no maxima at λ higher than 240 nm, but a slight shoulder around 280 nm, in agreement with those of the hydrolyzable tannins based on an acyclic glucose core and a nonahydroxytriphenoyl group (*30*). In accordance with mass spectra data in **Table 3**, these compounds were designated as the monomers vescalagin (peak 48) and castalagin (50), the pentosylated monomers grandinin (46) and roburin E (49), and the dimers roburin A (45) and D (47). Both first show a deprotonated molecule at *m/z* 933 and also gave ions at *m/z* 631 and 301 due to the loss of ellagic acid and to ellagic acid residue. The difference between their MS is provided by the ions at *m/z* 915 and 613, corresponding to $[M-H-H_2O]^-$ and [M − H− ellagic acid − H_2O]$^-$, which only occurs in vescalagin MS. In agreement with Quideau et al. (*31*) and Moilanen and Salminen (*32*), this type of water loss in ESI-MS is characteristics

Table 4. Global Valuations of Phenolic Compounds in *Castanea sativa* Heartwood[a]

	seasoned	light toasted	medium toasted
total polyphenols (mg gallic acid equivalent/g wood)	40.8 ± 9.21	25.9 ± 1.15	9.12 ± 0.92
total ellagitannins (mg ellagic acid released/g wood)	18.2 ± 2.28	8.33 ± 0.05	0.47 ± 0.13
total gallotannins (mg gallic acid released/g wood)	7.79 ± 3.35	2.19 ± 0.55	0.10 ± 0.008
total condensed tannins (mg (+)-catechin/g wood)	1.17 ± 0.12	nd	nd

[a] nd = not detected.

of vescalagin-type ellagitannins with the C-1 OH group of the glucose at the R_1 position, and not at R_2, as in castalagin-type ellagitannins. The quasimolecular ions of grandinin and roburin E (m/z 1065) were not very intense, and those of roburin A and D (m/z 1849) were not found. However, all of them gave an intense ion at m/z 301 [ellagic acid − H]$^-$. Roburin A and D also gave the [vescalagin − H]$^-$, [vescalagin − H − H_2O]$^-$, [M − 2H]$^{2-}$ and [M − 3H]$^{3-}$ fragments. All these identities were confirmed by comparing the retention times and UV and MS spectrum with those of the standards, kindly provided by Dr. A. Scalbert (France). Viriot et al. (*10*) found all these compounds in *Castanea sativa* Mill. heartwood, and using HPLC/LSIMS, vescalagin, castalagin, and roburin A were identified and quantified in chestnut heartwood by Vivas et al. (*33*). Some of them were detected in chestnut-derived commercial tannin extracts (*21*).

Peaks 42 and 43 show a deprotonated molecule at m/z 631 and were tentatively identified as vescalin and castalin, respectively. The elution order of these compounds was assigned taking into account data in literature (*34*), as well as the presence of a fragment at m/z 613 only in vescalin MS, corresponding to the loss of water from an ellagitannin with the C-1 OH group of the glucose at the R_1 position instead of at R_2, as occurs in vescalagin-type ellagitannins. These compounds are present in chestnut-derived commercial tannin extracts (*11*, *21*), but they are identified for the first time in chestnut wood, in addition to castalagin and vescalagin, by Mayer et al. (*35*). Peak 51 was also tentatively identified as acutissimin: its MS gave a deprotonated molecule at m/z 1205, as well as fragments at m/z 915, 613, and 301, like Saucier et al. (*36*) showed for acutissimin A and B. They also obtain an intense ion at m/z 602, but only in the MS of reference compound, and not when an aged-in-oak red wine extract was analyzed by HPLC-ESI/MS. The acutissimin A was also identified in *Castanea sativa* Mill. bark by Lampire et al. (*37*). Last, peak 44 remains unidentified. It shows the same UV spectrum, and its most intense ion in MS was at m/z 301, characteristic of an [ellagic acid − H]$^-$ fragment. Its MS also gave the [vescalagin/castalagin − H]$^-$ ion, and the 180 amu between the ions at m/z 481 and 301, which could be explained by the consecutive losses of glucosyl (162 amu) and water (18 amu) residues. No further identification was possible.

In **Figure 1**, we can also see three peaks, which were identified using commercial standards as 5-hydroxymethylfurfural, furfural, and 5-methylfurfural, on the basis of their retention times and UV spectra, and named in chromatogram as HMF, F, and MF, respectively. Because they are not phenolic compounds, they are not given further consideration here.

Phenolic Compounds in Seasoned and Toasted Chestnut Wood. To have an overall impression of the phenolic composition of seasoned and toasted chestnut wood, we carried out some global evaluations, summarized in **Table 4**. These data should be used with caution because they are obtained from spectrophotometric measures of the product of chemical reactions and should only be compared with data obtained in the same mode. The results imply that an important modification in phenolic composition, provoked by toasting in cooperage, takes place. Seasoned chestnut wood is characterized by its richness in hydrolyzable tannins, which are of the gallotannin and ellagitannin type, and a very low level of condensed tannins. Toasting causes the degradation of condensed tannins to levels lower than are possible to detect using this method, as well as a decrease in total polyphenols, which is likely related to the decrease in hydrolyzable tannins, being the intensity of decrease related to the intensity of toasting. However, other phenolic compounds must be formed during toasting because the decrease of total polyphenols was smaller than could be accounted for otherwise. As can be seen, the tannin composition of chestnut heartwood resembles that of oak used in cooperage because in oak high concentrations of hydrolyzable tannins are found (*4*−*6*, *30*) and toasting causes degradation of them as well. However, some differences were detected because in oak heartwood the hydrolyzable tannins were only of the ellagitannin kind because ellagic acid was the only compound produced in methanolysis of oak extracts. On the other hand, the highest levels of Folin−Ciocalteu index was found in seasoned chestnut heartwood, when compared to either seasoned or toasted oak.

A more detailed knowledge of the phenolic composition of this wood was obtained with HPLC analysis, as we can see in **Table 5**. Regarding low molecular weight (LMW) phenolic compounds, it highlights the concentrations of gallic and ellagic acids, as much in seasoned as in toasted wood, but while ellagic acid increases in relation to toasting intensity, gallic acid increases at the lowest intensity of toasting, but decreases at the most intense toasting. This increase of ellagic acid results from ellagitannin degradation (*4*−*6*), and the same should be true for gallotannins and gallic acid at the lowest intensity of toasting. However, gallic acid is so extremely sensitive to heat treatment that its content systematically and quickly decreases in the wood with the duration of toasting (*4*). Although we did not find data on toasted chestnut wood, these results were as expected if we take into account published data of brandies aged in chestnut barrels with three toasting intensities (*14*), in those that ellagic acid increases with toasting intensity, while gallic acid increases between light and medium toasting but decreases at strong toasting. Comparing the LMW phenolic composition of chestnut heartwood with that of oak wood, seasoned and toasted, chestnut shows some differences: the presence of protocatechuic acid and aldehyde and much higher amounts of gallic acid. These high levels of gallic acid in chestnut in relation to oak were also found by other authors when it comes to seasoned wood (*7*, *14*), but no data was found on toasted chestnut wood. This was additionally confirmed by higher levels of this compound in beverages aged in chestnut barrels compared to those aged in oak barrels. Thus, Canas et al. (*38*) found that brandy aged in chestnut barrels was richer in gallic acid than brandies aged in oak barrels from Portugal, France, and North America. Moreover, the red wine vinegars acetified in chestnut barrels can differ easily from those acetified in barrels of acacia, cherry, and oak because of their concentrations of gallic acid and gallic ethyl ester (*2*). Also Salagoity-Auguste et al. (*9*) and Vivas et al. (*11*) found higher levels of gallic acid in chestnut than in oak commercial tannins. These high levels of gallic acid and its ethyl ester could have an effect on the organoleptic characteristics of beverages because they have

Table 5. HPLC-DAD Quantitative Evaluation of Phenolic Compounds in Seasoned and Toasted Chestnut Heartwood[a]

peak	compound	(μg/g wood) seasoned	light toasted	medium toasted
	LMW Phenolic Compounds			
1	gallic acid	6166 ± 2359	8211 ± 61.3	2361 ± 144
2	protocatechuic acid	4.73 ± 1.22	7.39 ± 1.05	14.05 ± 0.85
10	vanillic acid	7.11 ± 3.76	28.8 ± 0.59	77.5 ± 4.71
14	syringic acid	7.38 ± 2.55	51.2 ± 7.53	152 ± 9.25
27	ferulic acid	10.4 ± 3.95	28.9 ± 2.70	6.05 ± 0.37
39	ellagic acid	588 ± 77	1406 ± 113	1801 ± 110
3	protocatechualdehyde	0.62 ± 0.41	6.74 ± 0.56	7.90 ± 0.48
12	vanillin	20.5 ± 6.63	163 ± 1.77	158 ± 9.61
19	syringaldehyde	14.0 ± 3.92	264 ± 16.5	374 ± 22.7
30	coniferaldehyde	8.42 ± 1.42	337 ± 11.7	328 ± 20.0
32	sinapaldehyde	11.8 ± 2.69	1219 ± 26.5	1230 ± 74.8
26	scopoletin	1.26 ± 1.14	6.73 ± 1.13	16.7 ± 2.23
40	unknown	nd	161 ± 5.03	1524 ± 92.7
∑	*LMW phenolic compounds*	*6849 ± 2393*	*11891 ± 111*	*8033 ± 489*
	Hydrolyzable Tannins Galloyl and Hexahydroxydiphenoyl Derivatives			
7	methyl gallate	144 ± 96.8	86.4 ± 7.25	nd
8	digalloyl glucose	139 ± 29.0	nd	nd
4	digalloyl-HHDP-glucose	36.7 ± 15.6	nd	nd
5	digalloyl-HHDP-glucose	110 ± 74.0	nd	nd
13	digalloyl-HHDP-glucose	643 ± 142	119 ± 1.34	nd
17	digalloyl-HHDP-glucose	90.5 ± 43.4	nd	nd
11	trigalloyl glucose	566 ± 410	320 ± 22.4	nd
15	trigalloyl glucose	53.6 ± 14.1	nd	nd
18	trigalloyl glucose	30.1 ± 22.5	nd	nd
20	trigalloyl glucose	1805 ± 1075	1422 ± 1.62	nd
22	trigalloyl glucose	1389 ± 989	nd	nd
25	trigalloyl-HHDP-glucose	98.3 ± 56.5	nd	nd
21	tetragalloyl glucose	196 ± 94.4	nd	nd
23	tetragalloyl glucose	222 ± 139	nd	nd
24	tetragalloyl glucose	53.6 ± 20.9	nd	nd
29	tetragalloyl glucose	3270 ± 1351	273 ± 18.9	nd
31	tetragalloyl glucose	143 ± 86.9	nd	nd
33	tetragalloyl glucose	654 ± 323	261 ± 13.8	nd
35	tetragalloyl glucose	97.3 ± 75.4	nd	nd
36	pentagalloyl glucose	2055 ± 970	1293 ± 12.0	nd
38	galloyl-valoneic acid dilactone	55.8 ± 37.4	nd	nd
6	unknown	29.2 ± 12.1	28.9 ± 0.42	28.5 ± 1.73
37	unknown	26.0 ± 35.1	243 ± 8.05	210 ± 12.8
∑	*galloyl and HHDP derivatives*	*11908 ± 5567*	*4047 ± 24.38*	*238 ± 14.5*
	Ellagic Derivatives			
9	ellagic acid deoxyhexose	37.4 ± 7.78	nd	nd
28	valoneic acid dilactone	250 ± 103	129 ± 1.55	nd
41	ellagic acid dimer dehydrated	82.9 ± 26.0	28.2 ± 1.97	nd
16	unknown	nd	10.5 ± 0.06	69.5 ± 4.23
34	unknown	40.6 ± 25.8	92.5 ± 3.58	193 ± 11.7
Σ	*ellagic derivatives*	*411 ± 123*	*260 ± 7.04*	*263 ± 16.1*
	Ellagitannins			
42	vescalin	1157 ± 482	144 ± 2	nd
43	castalin	1571 ± 716	758 ± 22	nd
48	vescalagin	15821 ± 5948	1025 ± 130	110 ± 3.50
50	castalagin	17373 ± 3667	6561 ± 81	547 ± 17.3
49	roburin E	2775 ± 640	877 ± 58	nd
46	grandinin	2370 ± 692	962 ± 63	nd
45	roburin A	2714 ± 1123	328 ± 30	nd
47	roburin D	2679 ± 793	401 ± 57	nd
51	acutissimin	3257 ± 1195	3900 ± 146	236 ± 7.46
44	unknown	751 ± 224	407 ± 5	nd
Σ	*ellagitannins*	*50469 ± 10809*	*15362 ± 206*	*894 ± 28.3*
Σ	*hydrolyzable tannins*	*62787 ± 10872*	*19668 ± 203*	*1395 ± 59*

[a] nd = no detected. HHDP = hexahydroxydiphenoyl. Galloyl and hexahydroxydiphenoyl derivatives were quantified as gallic acid, and ellagic derivatives as ellagic acid, in agreement with their UV profile. Vescalin and acutissimin were quantified as vescalagin, castalin as castalagin, and roburin D as roburin A.

recently been shown to be related to a puckering astringent mouth feel and bitterness and astringency, respectively, produced by red wine at taste thresholds lower than the concentrations detected in some beverages after their contact with chestnut wood (*39*).

Further, toasting produced, as expected, a lignin degradation that led to the formation of LMW phenolic compounds such as hydroxybenzoic acids, and especially hydroxybenzoic and hydroxycinnamic aldehydes, with higher amounts of 4-hydroxy-3,5-dimethoxy aldehydes, than 4-hydroxy-3-methoxy or 3,4-dihydroxy in toasted wood, and this lignin degradation was in relation to toasting intensity. In seasoned wood, vanillin was the most abundant aldehyde while in toasted wood sinapaldehyde was. Vanillin is the most important from an organoleptic point of view, in relation to the aging of wines, because it is an impact molecule with a vanilla smell. Its concentration, in both seasoned and toasted chestnut wood, was similar to that of other woods used in cooperage, inside the range of concentrations that can be expected for this compound (*4*, *6*). However, we found in the literature that when the same beverage (wine, vinegar, brandy) is aged in chestnut and oak barrels, the levels of vanillin detected in those aged in chestnut were higher than in those aged in oak (*2*, *3*, *38*).

Regarding hydrolyzable tannins, in seasoned wood we found a great variety (we quantified 38) with concentrations ranging between 2 and more than 25000 μg/g of wood, mainly from the ellagitannin family, except peak 44 (unidentified), which shows a significantly smaller concentration. The detected levels of vescalagin and castalagin, followed by A and E roburins, acutissimin and grandinin are especially important. Viriot et al. (*10*), also found that castalagin and especially vescalagin were the most abundant ellagitannins in heartwood from just felled chestnut trees, followed by roburin A. Moreover, four peaks (20, 22, 29, and 36), tentatively identified as tri, tetra, and pentagalloylglucose, were the most abundant of the gallotannins, followed by peaks 13, 11, and 33, tentatively identified as digalloyl-HHDP-, trigalloyl-, or tetragalloyl-glucose, with concentrations between 200 and 1400 μg/g of wood. After toasting, the concentration of tannins decreased by more than 70% at the lightest toasting and more than 95% at the most intense toasting, being ellagitannins the tannins more implied because while in seasoned wood they were the main compounds, with a total concentration of more than 50000 μg/g of wood, in the most intense toasted wood their quantification was only possible for three of them, vescalagin, castalagin, and acutissimin, showing concentrations of 110, 547, and 236 μg/g of wood, respectively. Similar results were obtained from the analysis of ellagitannins in seasoned and toasted Spanish, French, Portuguese, and American oak woods, showing the decrease of their contents, and an accentuation of this effect with the increasing treatment time and temperature and even an elimination of the ellagitannins from the surface layers of wood was observed (*4*, *5*, *40*). However, the hydrolyzable tannins found in oak heartwood were only of ellagitannin kind, being also the

monomers the most abundant, especially the nonpentosylated castalagin and vescalagin. The detected levels of ellagitannins in seasoned and light toasted oak heartwood were lower than in seasoned and light toasted chestnut heartwood, but their decrease at more intense toasting led to similar levels in both oak and chestnut, as happened in some oaks species which showed high levels of ellagitannins at the end of seasoning (*Q. robur, Q. pyrenaica*) (*4*, *5*, *40*).

As we have seen, the toasting conditions applied provoked an important modification in the phenolic composition of chestnut wood and, therefore, in the organoleptic characteristics of beverages treated with this wood. Thus, toasting causes the degradation of hydrolyzable tannins, as well as a degradation of lignin with the resulting increase in lignin constituents, provoking also a decrease of natural quantitative variability as can be expected by taking into account data in literature (*5*, *6*). As happens in oak wood, in the lightest toasting processes, the degradation of wood components was minor and the toasted wood obtained showed different balance pattern between hydrolyzable tannins and LMW phenolic compounds related to toasting intensity. On the other hand, the products of the heat degradation of hydrolyzable ellagitannins, such as dehydrocastalagin, deoxyvescalagin, deoxyroburin A, or dehydroroburin D, were not found in the toasted chestnut wood we studied. These compounds, which impart a mouth-coating and astringent oral sensation with relatively low threshold concentration, were identified when the pure compounds were thermally treated in model experiments, but as far as we know, they have still not been found in toasted wood (*41*).

Taking oak wood as a reference, in the interaction process between chestnut wood and the different kinds of beverages (wines, spirits, vinegars, ciders, etc.), some aspects of its phenolic composition should be borne in mind. If intense toasted barrels are used, chestnut wood will provide the same phenolic compounds as oak, together with small quantities of protocatechuic acid and aldehyde, and relatively high quantities (between 28 and 1500 μg/g of wood) of some unidentified compounds (peaks 6, 16, 34, 37, and 40). Among all these compounds, the high levels of gallic acid could have an important effect on the organoleptic characteristics of beverages. Moreover, different toasting levels will provoke different balances between tannins and lignin constituents, thus the impact of this wood on aging different beverages will be related to toasting intensity. In lightly toasted and untoasted barrels, the tannins provided will also be hydrolyzable, as in oak, but including both ellagitannins and gallotannins, the latter not being found in oak wood. Therefore it is not known what the possible implications in the chemical modifications that take place during beverage aging, as well as in their organoleptic characteristics, might be. As far as we know, this is the first time some of these gallotannins and ellagic derivatives have been identified in chestnut heartwood.

ACKNOWLEDGMENT

This study was financed by Tonelería Intona, SL, and Navarra Government (Project: "Caracterización de maderas alternativas al roble en tonelería para uso alimentario") and Ministerio de Ciencia e Innovación (Project RTA2009-0046). Miriam Sanz received a contract from the Spanish Government through the *Torres Quevedo* program. We thank Mr. Antonio Sánchez for his help throughout the chemical analysis.

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Received for review July 13, 2010. Revised manuscript received July 26, 2010. Accepted July 27, 2010.