Phenolic Compounds in Cherry (Prunus avium) Heartwood with a View to Their Use in Cooperage

ARTICLE *in* JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · MARCH 2010

Impact Factor: 2.91 · DOI: 10.1021/jf100236v · Source: PubMed

7 AUTHORS, INCLUDING:

Estrella Cadahía
Instituto Nacional de Investigación y Tecnol…
75 PUBLICATIONS **1,346** CITATIONS
SEE PROFILE

Brigida Fernández de Simón
Instituto Nacional de Investigación y Tecnol…
66 PUBLICATIONS **1,479** CITATIONS
SEE PROFILE

Isabel Estrella
Spanish National Research Council
40 PUBLICATIONS **1,184** CITATIONS
SEE PROFILE

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 4907–4914 4907
DOI:10.1021/jf100236v

Phenolic Compounds in Cherry (*Prunus avium*) Heartwood with a View to Their Use in Cooperage

Miriam Sanz,[†] Estrella Cadahía,[‡] Enrique Esteruelas,[†] Ángel M[A] Muñoz,[†] Brígida Fernández De Simón,*[,‡] Teresa Hernández,[§] and Isabel Estrella[§]

[†]Departamento I+D+I, Industrial Tonelera Navarra (INTONA), Polígono "La Moyuela", Monteagudo (Navarra), Spain, [‡]Departamento de Productos Forestales, Centro de Investigación Forestal (CIFOR), Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Apdo. 8111, 28080 Madrid, Spain, and [§]Instituto de Fermentaciones Industriales (CSIC), Juan de la Cierva, 3, 28006 Madrid, Spain

The phenolic and tannic composition of heartwood extracts from *Prunus avium*, commonly known as cherry tree, before and after toasting in cooperage were studied using HPLC-DAD and HPLC-DAD/ESI-MS. Nonflavonoid (16 compounds) and flavonoid (27 compounds) polyphenols were identified, 12 of them in only a tentative way. The nonflavonoids found were lignin constituents, and their pattern is different compared to oak, since they include compounds such as protocatechuic acid and aldehyde, *p*-coumaric acid, methyl vanillate, methyl syringate, and benzoic acid, but not ellagic acid, and only a small quantity of gallic acid. In seasoned wood we found a great variety of flavonoid compounds which have not been found in oak wood for cooperage, mainly, in addition to the flavan-3-ols (+)-catechin, a B-type procyanidin dimer, and a B-type procyanidin trimer, the flavanones naringenin, isosakuranetin, and eriodictyol and the flavanonols aromadendrin and taxifolin. Seasoned and toasted cherry wood showed different ratios of flavonoid to nonflavonoid compounds, since toasting results in the degradation of flavonoids, and the formation of nonflavonoids from lignin degradation. On the other hand, the absence of hydrolyzable tannins in cherry wood, which are very important in oak wood, is another particular characteristic of this wood that should be taken into account when considering its use in cooperage.

KEYWORDS: *Prunus avium*; cherry; heartwood; tannins; phenolic compounds; toasting

INTRODUCTION

Although oak (*Quercus* spp.) heartwood is the main material used in making containers in cooperages, other species such as chestnut (*Castanea sativa*), cherry (*Prunus avium*), acacia (*Robinia pseudoacacia*), and, more rarely, ash (*Fraxinus excelsior* and *Fraxinus vulgaris*) and mulberry (*Morus alba* and *Morus nigra*) have been considered as possible sources of wood, for production of both wines and their derived products, such as vinegar, cider, and spirits such as brandies (*1*−*5*), although only oak and chestnut are approved by the OIV. Various producers even prefer using local woods in order to reduce cost, and recently some wine cellars have ordered barrels with some nonoak staves included from cooperages. During aging the beverages undergo 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.

The impact that woods different from oak have on the chemical composition and sensorial properties of beverages has not yet been fully evaluated. As a step toward this goal, the characteristics of wines and especially vinegars aged in barrels made of different woods have been studied very recently (*4*−*6*), finding in some cases compounds that could act as markers of the use of a certain wood. However, for a more complete evaluation of the impact of these woods, more information about their chemical composition and physical−mechanical properties as well as of their evolution during seasoning and toasting at cooperage is necessary, similarly to studies done in the last 20 years regarding oak wood for cooperage needs. In fact, some papers have recently been published about the volatile composition of woods other than oak, with a view to their use in barrel making (*7*−*9*).

One of the woods considered as a possible alternative to oak is cherry wood. Cerezo et al. (*4*) found that in red wine vinegars produced by surface culture in barrels made of different woods (oak, chestnut, acacia, and cherry), oak and cherry gave the highest scores for most of the descriptors, analyzed after acetification, with the very highest being those obtained in red fruit attributes and general impression notes. Moreover, the discriminant analysis of phenolic compounds in these vinegars was able to classify the samples according to the kind of wood used in their production. De Rosso et al. (*5*) also found some special characteristics in wines aged in one particular untoasted barrel of cherry wood in comparison with other woods (acacia, chestnut, mulberry, and oak), suggesting higher oxygen permeation

*To whom correspondence should be addressed. Tel: 34-913476789. Fax: 34-913476767. E-mail: fdesimon@inia.es.

Published on Web 03/22/2010

through their staves; therefore, they propose their use for shorter aging times. However, Torija et al. (*6*) found the highest oxygen permeation not in cherry wood barrels used in traditional vinegar production but in acacia wood barrels, and only when the vinegars were produced in a factory in the open air and subjected to dramatic changes in temperature. As can be seen, the impact that cherry wood has on the chemical composition and sensorial properties of beverages requires further study, and research into its chemical composition will help toward that goal.

The few published studies on the chemical composition of *Prunus avium*, or cherry tree wood, point out important chemical differences when it is compared to oak woods that should be kept in mind when considering woods for cooperage. The oak heartwood shows high levels of ellagitannins, benzoic and cinnamic acid 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 were the monomer ellagitannins, castalagin, roburin E, vescalagin, and grandinin, and low-molecular-weight phenolic compounds, ellagic and gallic acids, in addition to lignin derivatives, especially vanillin. It also provides a great deal 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, which means it is a balanced wood, since it can do so without masking the wine primary and secondary aromas (*10*, *11*). Oak heartwood does not contain flavonoid compounds. However, in cherry heartwood from recently felled 70-year-old trees, Vinciguerra et al. (*12*) identified five flavanones (pinocembrin, pinostrobin, dihydrowogonin, naringenin, and sakuranetin), one dihydroflavonol (aromadendrin-7-methyl ether), and two flavones (chrysin and tectochrysin) from the diethyl ether fraction of the acetone/water extract, using GC-MS. More recently, McNulty et al. (*13*) isolated tectochrysin, sakuranetin, dihydrowogonin, naringenin, aromadendrin, and catechin from the heartwood of branches (4−8 years of age) from mature orchard-cultivated specimens of *P. avium*. In addition, using GC-MS to analyze 50% hydroalcoholic and model wine extracts, many volatile compounds were identified in seasoned (*7*, *9*) and toasted cherry heartwood, 98 being quantified, among them lignin and carbohydrate and lipid derivatives (*8*). In comparison to oak wood, cherry heartwood contains not only relatively large amounts of flavonoid compounds but also high concentrations of some volatile compounds, such as methyl syringate, 3,4,5-trimethoxyphenol, benzoic acid, benzyl salicylate, and methyl vanillate in seasoned and toasted wood and low levels of phenyl aldehydes, as well as low levels of carbohydrate and lipid derived volatile compounds, especially in toasted wood.

The objective of this work is to study the tannic and polyphenolic composition of cherry (*Prunus avium*) heartwood and their 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, using oak wood as a reference. This and other woods could be 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 pieces are used, but these other woods could be considered in order to give a particular personality to the products.

MATERIALS AND METHODS

Wood Samples. Cherry (*Prunus avium*) heartwood was provided as staves for making barrels by Tonelería Intona, SL (Navarra, Spain). The wood was naturally seasoned for 24 months and toasted at medium intensity (185 °C for 45 min), in an industrial kiln specially designed for toasting staves. Samples were taken before and after toasting, five 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. The samples were mixed, because our objective was to study the general phenolic profile of this wood both before and after toasting, without going deeply into natural variation.

Chemicals. Reference compounds were obtained from commercial sources: pinostrobin, gallic acid, and protocatechualdehyde (Fluka Chimie AG, Buchs, Switzerland), protocatechuic acid, *p*-hydroxybenzoic acid, methyl vanillate, methyl syringate, 3,4,5-trimethoxyphenol, syringaldehyde, coniferyl aldehyde, and scopoletin (Aldrich Chimie, Neu-Ulm, Germany), aromadendrin, sakuranetin, tectochrysin, pinocembrin, and ellagic acid (Apin, Oxon, U.K.), (+)-catechin, (−)-epicatechin, vanillin, benzoic acid, syringic acid, chrysin, taxifolin, quercetin, and hesperetin (Sigma Chemical, St. Louis, MO), eriodictyol (Roth, Karlsruhe, Germany), and procyanidin B1 and B2, cyanidin chloride, vanillic acid, sinapaldehyde, naringenin, kaempferol, apigenin, isosakuranetin, butein, prunin, and kaempferide (Extrasynthèse, Genay, France). Methanol, diethyl ether, ethyl acetate, anhydrous sodium sulfate, and phosphoric acid were purchased from Panreac (Barcelona, Spain). Acetic acid and methanol HPLC grade were obtained 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 and in darkness for 24 h. The extracts were filtered in a Büchner funnel. After removal of the methanol in a rotary evaporator at a temperature below 40 °C, the aqueous solution (solution I) was extracted with diethyl ether and ethyl acetate and then the remainder of the aqueous solution was freeze-dried. The organic fractions were dried and redissolved in methanol. These extracts, in addition to an aliquot part of freeze-dried extract redissolved in water, were used for the HPLC-DAD quantitative determination of low-molecular-weight phenolic compounds and flavonoids, and the ethyl acetate and freeze-dried extracts were used for the global quantitation of tannins. The extracts were studied in duplicate.

Global Valuation. In solution I, total polyphenols were determined by the Folin−Ciocalteau assay with gallic acid as standard (*14*). In the ethyl acetate and freeze-dried extracts condensed tannins were determined by the vanillin method with (+)-catechin as standard (*15*), and hydrolyzable tannins by HPLC quantification of gallic and ellagic acid released after acid methanolysis (*16*). A second global valuation of condensed tannins was carried out by HPLC quantification of anthocyanidins released after acid butanolysis (*17*). All analyses 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, 280, 325, 340, and 525 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) to 85% (20 min), 75% (30 min), 50% (50 min), and 0% (70 min), using methanol/0.1% phosphoric acid as eluent B. The total time of analysis was 70 min, the equilibration time was 10 min, and the flow rate was 1 mL/min. With the same eluents, the elution gradient to analyze the freeze-dried sample (30 mg) was as follows: 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 280 nm for flavan-3-ols and at 325 nm for the remaining compounds. The concentration of each substance was measured by comparing it with calibrations made with the pure compound analyzed under the same conditions ($r^2 \geq 0.988$). Unidentified flavonoids were quantified as aromadendrin (peak 27), naringenin (peaks 32 and 38), and butein (peak 33). Unidentified procyanidins were quantified as procyanidin B2. 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.

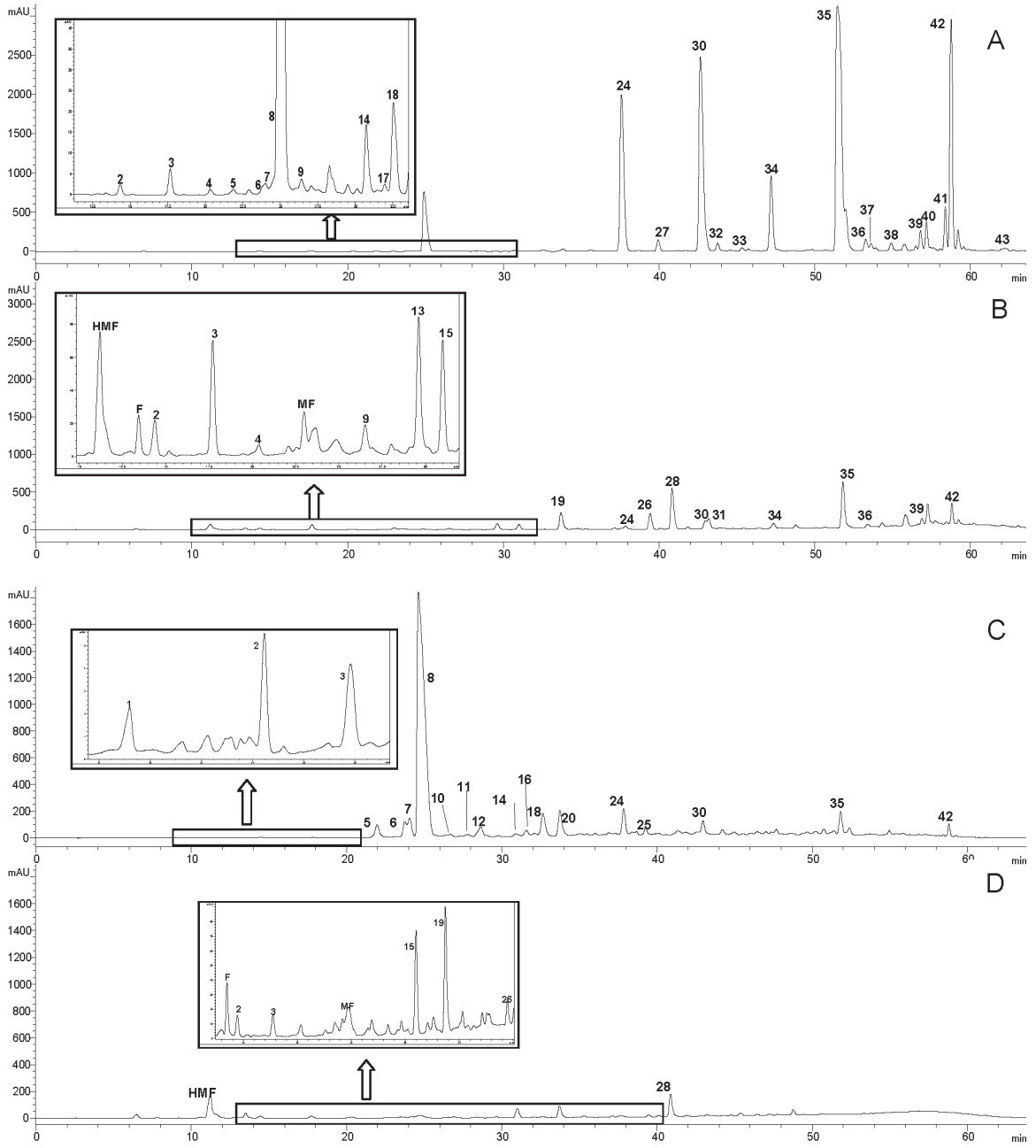

Figure 1. HPLC-DAD chromatograms of *Prunus avium* heartwood extracts, monitored at 280 nm: (**A**) diethyl ether extract of untoasted heartwood; (**B**) diethyl ether extract of toasted heartwood; (**C**) ethyl acetate extract of untoasted heartwood; (**D**) ethyl acetate extract of toasted heartwood. Peak numbers are as in **Tables 1** and **2**. The peaks have been numbered in agreement with retention times, using the same number in the different chromatograms for the same compound.

The binary mobile phase consisted of solvents A (2% acetic acid in HPLC grade water) and B (HPLC grade methanol). The column, gradient, volume injection, and temperature of the analytical column were the same as those 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; nebulizer pressure; 55 psi (380 Pa); capillary voltage, 4000 V. Mass spectra were acquired using electrospray ionization in the negative mode at the voltage gradients 80 V fragmentation voltage for m/z 0−200 and 200 V fragmentation voltage for m/z 200−3000 and recorded for the range of m/z 100−3000.

RESULTS AND DISCUSSION

Identification of Phenolic Compounds. Figure 1 illustrates the HPLC chromatograms of the phenolic compounds of diethyl ether and ethyl acetate extracts from seasoned and toasted cherry heartwood. As can be seen, the four chromatograms show qualitative and especially quantitative differences, related to both the kind of extract and the processing the wood underwent (seasoned or toasted). Chromatograms from freeze-dried extracts were not shown because of the total absence of peaks in toasted wood and the presence of only four peaks in seasoned wood, all of which were also detected in ethyl acetate extract. A total of 31 flavonoid and nonflavonoid compounds were identified by comparing their retention times and UV and mass spectra with those of the standards. In addition, 12 peaks corresponding to compounds with related structures were tentatively identified on the basis of their retention time, UV spectra, and MS patterns, as well as taking into account data in related literature. Eight of them show spectroscopic and spectrometric characteristics of flavan-3-ol, one of a

Table 1. Spectroscopic and Spectrometric Data of Non-Flavonoid Compounds in Seasoned and Toasted *Prunus avium* Heartwood[a]

peak	R_t	compd	λ_{max}	$[M-H]^-$	negative ion *m/z* (% in MS) [attribution]
1	9.2	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) $[M-H-CO_2]^-$
3	17.7	protocatechualdehyde	280, 310	137	137 (100) $[M-H]^-$
4	20.4	*p*-hydroxybenzoic acid	256	137	137 (100) $[M-H]^-$
9	26.5	vanillic acid	260, 292	167	167 (100) $[M-H]^-$; 152 (34) $[M-H-CH_3]^-$
13	29.0	vanillin	280, 312	151	151 (100) $[M-H]^-$; 136 (86) $[M-H-CH_3]^-$
15	31.0	syringic acid	274	197	197 (100) $[M-H]^-$; 153 (23) $[M-H-CO_2]^-$
17	32.5	3,4,5-trimethoxyphenol	276	183	183 (17) $[M-H]^-$; 168 (94) $[M-H-CH_3]^-$; 153 (100) $[M-H-2CH_3]^-$
19	33.7	syringaldehyde	232 sh, 308	181	181 (100) $[M-H]^-$; 166 (61) $[M-H-CH_3]^-$; 151 (31) $[M-H-2CH_3]^-$
21	34.8	benzoic acid	230	121	121 (100) $[M-H]^-$
22	35.5	*p*-coumaric acid	290 sh, 310	163	163 (42) $[M-H]^-$; 119 (100) $[M-H-CO_2]^-$
23	37.6	scopoletin	258 sa, 294 sh, 342	191	191 (55) $[M-H]^-$; 176 (100) $[M-H-CH_3]^-$
26	39.4	coniferaldehyde	290 sh, 322	177	177 (100) $[M-H]^-$;162 (41) $[M-H-CH_3]^-$
28	40.8	sinapaldehyde	300 sh, 338	207	207 (36) $[M-H]^-$; 192 (100) $[M-H-CH_3]^-$
29	41.3	methyl vanillate	262, 296	181	181 (92) $[M-H]^-$; 166 (100) $[M-H-CH_3]^-$
31	43.2	methyl syringate	276	211	211 (100) $[M-H]^-$; 196 (70) $[M-H-CH_3]^-$

[a] R_t expressed in min, λ_{max} in nm, and $[M-H]^-$ in *m/z*.

Table 2. Spectroscopic and Spectrometric Data of Flavonoid Compounds in Seasoned and Toasted *Prunus avium* Heartwood[a]

peak	R_t	compd	λ_{max}	$[M-H]^-$	negative ion *m/z* (% in MS) [attribution]
			Procyanidins		
5	22.0	B-type procyanidin dimer	280	577	577 (100) $[M-H]^-$; 425 (22) $[M-C_8H_8O_3-H]^-$; 407 (35) $[M-C_8H_8O_3-H_2O-H]^-$; 289 (50) $[M_B-H]^-$; 137 (77) $[^{1,3}B]^-$
6	23.7	procyanidin B1[b]	280	577	577 (100), 425 (18), 407 (35), 289 (37)
7	24.1	B-type procyanidin trimer	280	865	865 (25) $[M-H]^-$; 713 (10) $[M-C_8H_8O_3-H]^-$; 577 (100) $[M-M_B-H]^-$; 425 (20) $[M-M_B-C_8H_8O_3-H]^-$; 407 (20) $[M-M_B-C_8H_8O_3-H_2O-H]^-$; 289 (58) $[M_B-H]^-$;
8	24.6	(+)-catechin	278	289	289 (100) $[M-H]^-$; 179 (15) $[^{1,2}A]^-$; 151 (10) $[^{1,3}A]^-$; 137 (30) $[^{1,3}B]$; 125 (5) $[^{1,4}A]^-$; 109 (7) $[^{1,2}B]^-$
10	26.7	B-type procyanidin dimer[b]	280	577	577 (90), 425 (22), 407 (35), 289 (100)
11	27.8	B-type procyanidin trimer[c]	280	865	865 (37), 713 (25), 577 (90), 425 (47), 407 (43), 289 (87)
12	28.6	procyanidin B2[b]	280	577	577 (100), 425 (27), 407 (30), 289 (35)
14	30.9	B-type procyanidin dimer[b]	277	577	577 (100), 425 (18), 407 (35), 289 (37)
16	32.0	B-type procyanidin dimer[b]	280	577	577 (65), 425 (28), 407 (20), 289 (100),
18	32.6	(−)-epicatechin	280	289	289 (100) $[M-H]^-$; 245 (33) $[M-H-CO_2]^-$; 151 (15) $[^{1,3}A]^-$
20	33.7	B-type procyanidin dimer[b]	280	577	577 (100), 425 (27), 407 (28), 289 (30)
25	39.3	B-type procyanidin dimer[b]	278	577	577 (90), 425 (22), 407 (35), 289 (100)
			Other Flavonoids		
24	37.6	taxifolin	290, 336 sh	303	303 (100) $[M-H]^-$; 179 (36) $[^{1,2}A]^-$; 177 (38) $[^{1,4}B]^-$; 151 (20) $[^{1,3}A]^-/[^{1,3}B]^-$; 125 (73) $[^{1,4}A]^-$
27	39.9	tetrahydroxy flavanonol	292, 346 sh	303	303 (34) $[M-H]^-$; 179 (18) $[^{1,2}A]^-$; 177 (39) $[^{1,4}B]^-$; 151 (18) $[^{1,3}A]^-/[^{1,3}B]^-$; 125 (100) $[^{1,4}A]^-$
30	42.6	aromadendrin	292, 338 sh	287	287 (100) $[M-H]^-$; 259 (9) $[M-H-CO]^-$; 179 (16) $[^{1,2}A]^-$; 151 (24) $[^{1,3}A]^-$; 125 (65) $[^{1,4}A]^-$
32	43.8	tetrahydroxy flavanone	288, 334 sh	287	287 (60) $[M-H]^-$; 151 (26) $[^{1,3}A]^-$; 125 (100) $[^{1,4}A]^-$
33	45.4	tetrahydroxy chalcone derivative	280, 390	575	575 (100); 394 (66); 271 (23);
34	47.2	eriodictyol	288, 334 sh	287	287 (28) $[M-H]^-$; 151 (100) $[^{1,3}A]^-$; 135 (22) $[^{1,3}B]^-$
35	51.4	naringenin	290, 332 sh	271	271 (65) $[M-H]^-$; 177 (12) $[M-H-B\ ring]^-$; 151 (100) $[^{1,3}A]^-$; 119 (17) $[^{1,3}B]^-$
36	53.3	quercetin	254, 372	301	301 (100) $[M-H]^-$; 299 (14) $[M-H-H_2]^-$; 179 (14) $[^{1,2}A]^-$; 151 (37) $[^{1,3}A]^-$; 121 (6) $[^{1,2}B]^-$
37	53.6	hesperetin	288, 332 sh	301	301 (100) $[M-H]^-$; 286 (23) $[M-H-CH_3]^-$; 151 (28) $[^{1,3}A]^-$
38	54.9	trihydroxy flavanone	288, 338 sh	271	271 (100) $[M-H]^-$; 151 $[^{1,3}A]^-$
39	56.8	kaempferol	264, 366	285	285 (100) $[M-H]^-$; 283 (8) $[M-H-H_2]^-$; 193 (12) $[M-H-B\ ring]^-$; 177 $[^{0,4}B]^-$
40	57.6	apigenin	268, 338	269	269 (100) $[M-H]^-$
41	58.3	pinocembrin	255, 332 sh	255	255 (100) $[M-H]^-$
42	58.7	isosakuranetin	290, 332 sh	285	285 (100) $[M-H]^-$
43	62.0	kaempferide	266, 366	299	299 (52) $[M-H]^-$; 271 (100) $[M-H-CO]^-$; 284 (63) $[M-H-CH_3]^-$; 255 (35) $[M-H-CO_2]^-$; 227 (22) $[M-H-C_2O_3]^-$; 193 (20) $[M-H-B\ ring]^-$; 151 (9) $[^{1,3}A]^-$

[a] R_t is expressed in min and λ_{max} in nm. [b] Attribution of ions of mass spectrum as in peak 5. [c] Attribution of ions of mass spectrum as in peak 7.

chalcone derivative, one of flavanonol, and two of flavanone or flavanonol. Relevant information obtained from DAD and ESI-MS is shown in **Tables 1** and **2**: λ_{max} and shoulders if they exist from UV spectra, and fragment ions observed in negative ionization mode, their percentage in the MS, and the structure attribution of ions. In discussing the mass fragmentation of flavonoids, we have used the nomenclature proposed by Ma et al. (*18*) to describe the resulting fragment ions. The fragments $[^{i,j}A]^-$ and $[^{i,j}B]^-$ represent product ions containing intact A and B rings of the flavonoid skeleton; superscripts i and j indicate the C-ring bonds that had

been broken. Additionally, in the particular case of flavanol units in linear and branched proanthocyanidins, we have used the nomenclature developed by Porter (*19*), which depends on the positions of the interflavanoid bonds at A and C rings: a T-unit (top) has only one interflavonoid linkage at C-4, while an M − unit (middle) has an additional linkage at C-6 or C-8. If all three positions are involved in C−C linkages, the monomer is a J-unit. A monomer with one C−C bond at C-8 or C-6 is called a B-unit (base).

Some low-molecular-weight phenolic compounds, lignin derivatives, were identified (**Figure 1**, **Table 1**). Among these were the benzoic acids gallic (peak 1), protocatechuic (2), *p*-hydroxybenzoic (4), vanillic (9), syringic (15), and benzoic (21), the hydroxycinnamic acid *p*-coumaric (22), the hydroxybenzoic aldehydes vanillin (13) and syringaldehyde (19), the hydroxycinnamic aldehydes coniferylic (26) and sinapylic (28), the methyl esters of vanillic (29) and syringic (31) acids, the coumarin scopoletin (23), and 3,4,5-trimethoxyphenol (17). Except in the cases of *p*-coumaric acid, scopoletin, sinapaldehyde, methyl vanillate, and 3,4,5-trimethoxyphenol, the respective $[M - H]^-$ quasi molecular ion was the base peak in the MS pattern. The mass spectra of sinapaldehyde, scopoletin, and methyl vanillate also gave the deprotonated molecules $[M - H]^-$ at *m*/*z* 207, 191, and 181, respectively, although the main ion is due to the loss of a methyl group and, in the case of 3,4,5-trimethoxyphenol, of two methyl groups. Gallic, protocatechuic, syringic, and *p*-coumaric acids also gave the ion $[M - H - 44]^-$ fragment ion via loss of a CO_2 group from the carboxylic acid moiety, which in *p*-coumaric acid MS was the most prominent ion (*m*/*z* 119). The fragmentation of vanillic acid produced an anion radical with *m*/*z* 152 ($[M - H - 15]^-$) by losing a CH_3 group from the deprotonated molecular ion. In the methoxylated aldehydes vanillin and syringaldehyde, the sequential loss of CH_3 was observed to give fragments at *m*/*z* 136 and at *m*/*z* 166 and 151, respectively. All these identities were confirmed with the authentic standard.

To continue, peaks 5−8, 10−12, 14, 16, 18, 20, and 25 (**Figure 1**, **Table 2**) showed the typical UV profile of proanthocyanidins/catechin-like compounds with an absorbance maximum between 277 and 280 nm (*20*). The butanolysis of the extracts followed by HPLC-DAD analysis of released compounds showed the cyanidin as the only formed compound, indicating that only procyanidins are present in the extract, which is consistent with the MS results. Peaks 8 and 18 presented a molecular ion ($[M - H]^-$) at *m*/*z* 289, which indicated a structure of monomeric flavanol. Identification of these compounds as the monomers (+)-catechin and (−)-epicatechin was performed by comparing the retention time, fragmentation pattern, and UV spectrum with those of standards. Moreover, the MS analysis of peaks 5, 6, 10, 12, 14, 16, 20, and 25 showed a molecular ion at *m*/*z* 577, characteristic of B-type procyanidins (*20*). As expected for proanthocyanidins, retro Diels−Alder (RDA) fission of the heterocyclic rings of dimeric procyanidins occurred and resulted in the fragment *m*/*z* 425. The product of the subsequent water elimination (*m*/*z* 407) was also detected in significant amounts, sometimes greater than those of the RDA products. The cleavage of the interflavonoid linkage leading to *m*/*z* 289 $[M_B - H]^-$ was also observed, whereas the ion fragments at *m*/*z* 287 corresponding to $[M_T - 3H]^-$ was not detected. These ions further confirmed the structure of dimeric procyanidins of these peaks. Due to the lack of commercially available reference substances, only peaks 6 and 12 could be determined as procyanidins B1 and B2, respectively. Lastly, peaks 7 and 11 showed a quasi molecular ion at *m*/*z* 865, *m*/*z* 577 and *m*/*z* 289 being the main fragments obtained, which were thus tentatively identified as B-type procyanidin trimers (*20*).

The R_t values, UV/vis spectra, and MS fragmentation pattern of peaks 24 and 30 (**Figure 1**, **Table 2**) matched those of the flavanonol standards taxifolin (*m*/*z* 303) and aromadendrin (*m*/*z* 287). Peak 30 shows, under our chromatographic conditions, a retention time very similar to that of prunin (42.77 min), a flavanone glucoside identified in both the callus and the phloem of *P. avium* (*21*). However, neither its UV spectrum (λ_{max} 284 nm) nor its MS pattern ($[M - H]^-$ *m*/*z* 433) match with those of the peak at 42.65 min. Peak 27 (39.93 min) was also tentatively assigned to a flavanonol. The molecular weight of its $[M - H]^-$ ion (*m*/*z* 303) could belong to a tetrahydroxymethoxyflavanol, a pentahydroxyflavanone, or a tetrahydroxyflavanonol, but the UV/vis spectrum rules out the flavanol structure. The fragments *m*/*z* 125 and 151 were assigned to $[^{1,4}A]^-$ and $[^{1,3}A]^-$, respectively, which indicated the dihydroxy substitution of the A ring in either a flavanone or a flavanonol structure. The second and third most significant fragments, *m*/*z* 177 and 179, were assigned to $[^{1,4}B]^-$ and $[^{1,2}A]^-$; as far as the latter is concerned, the only possibility is a flavanonol structure, which establishes the B-ring as dihydroxylated (*22*). According to the above data peak 27 was identified as an A-dihydroxy-B-dihydroxyflavanonol.

Continuing on, peaks 34, 35, 37, 41, and 42 were identified as the flavanones eriodictyol, naringenin, hesperetin, pinocembrin, and isosakuranetin, respectively, by comparing their R_t values, UV/vis spectra, and MS fragmentation patterns with those of the standards. Naringenin and pinocembrin have already been isolated from the heartwood of recently felled trees of *P. avium* (*12*, *13*). However, other compounds found by these authors, such as pinostrobin, dihydrowogonin, sakuranetin, aromadendrin-7-methyl ether, chrysin, and tectochrysin, were not found in our samples, although the standards were analyzed under our conditions, except for dihydrowogonin and aromadendrin-7-methyl ether (unavailable commercial standards). Isosakuranetin and sakuranetin show very similar retention times (58.75 and 58.21 min, respectively), and λ_{max} values (290, 332 sh and 290, 334 sh nm, respectively), and the molecular weights of their $[M - H]^-$ ions (*m*/*z* 285) are the same. However, the percentages of these quasi molecular ions were different: 100% in isosakuranetin and 15% in sakuranetin, and when the sakuranetin standard was coinjected with the sample in the HPLC-DAD, an additional peak appeared in the chromatogram. This led us to assign the identity of peak 42 to isosakuranetin. On the other hand, two other peaks were tentatively identified as flavanone derivatives. Peak 32 (43.76 min) and peak 38 (54.90 min) showed the typical UV/vis spectrum of flavanones or flavanonols in which band I is reduced to little more than a shoulder, at 334 and 338 nm in these cases, and band II is the main one (at 288 nm) (*23*). In peak 32 the molecular weight of the $[M - H]^-$ ion (*m*/*z* 287) indicated that it could be a trihydroxyflavanonol or a tetrahydroxyflavanone. Fragments *m*/*z* 125 and 151 were assigned to $[^{1,4}A]^-$ and $[^{1,3}A]^-$, which indicates the dihydroxy substitution of the A-ring in the case of either a flavanone or a flavanonol structure. In peak 38, the ion with the highest relative abundance, $[M - H]^-$ ion (*m*/*z* 271), indicated that it could be a trihydroxyflavanone or a dihydroxyflavanonol. The fragment *m*/*z* 151 was assigned to $[^{1,3}A]^-$, which indicates the dihydroxy substitution of the A-ring in both cases (*22*). No further identification was possible.

Neither a complete identification was possible with peak 33 (48.66 min), which showed a UV/vis profile with an intense band I at 390 nm, suggesting a chalcone skeleton (*23*). Mass spectrometric data showed three ions with high relative abundance at *m*/*z* 575, 394, and 271, which also suggested a possible chalcone derivative. The *m*/*z* 271 ion suggests a possible tetrahydroxychalcone derivative.

Finally, three flavonols and one flavone were identified. Peaks 36, 39, and 43 were first distinguished by their UV profile, which shows an intense band I that lies between 350 and 385 nm (at 372,

Table 3. Global Valuations of Phenolic Compounds in *Prunus avium* Heartwood

	seasoned wood	toasted wood
total polyphenols (mg of gallic acid equiv/g of wood)	63.18	4.01
total ellagitannins (mg of ellagic acid/g of wood)	1.66	1.79
total procyanidins (mg of cyanidin released/g of wood)	218.70	nd

366, and 366 nm, respectively). This feature distinguishes flavonols from flavones, as in the latter case band I shows the highest intensity in the 310−350 nm range (*23*). These data together with those of retention time and MS pattern led us to identify these peaks as quercetin, kaempferol, and kaempferide, and this was confirmed with the standards. In the case of kaempferide, no confusion with pinostrobin was possible, because although retention times are very similar under our chromatographic conditions (62.6 min), and pinostrobin has already been isolated from the heartwood of recently felled trees of *P. avium* (*12*), their UV/vis spectrum (λ_{max} 290, 332 sh) and MS pattern ($[M - H]^-$ ion at m/z 270) are very different from those of peak 41. In the same way peak 40 was assigned to apigenin, by comparing it with the standard.

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

Phenolic Compounds in Seasoned and Toasted Cherry Wood. In order to gain an overall impression of the phenolic composition of seasoned and toasted cherry wood, we carried out some global valuations, summarized in **Table 3**. These data should be used with caution, because they are obtained from spectrophotometric measurements of the products of chemical reactions and should only be compared with data obtained in the same way. The results imply that 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 tannins. Seasoned cherry wood is characterized by its richness in condensed tannins, which are exclusively of the procyanidin type, since cyanidin was the only anthocyanidin found after butanolysis. In addition, very low levels of hydrolyzable tannins were found in this wood. On the other hand, toasted cherry wood showed low levels of total polyphenols and hydrolyzable tannins, and condensed forms were not detected at all. As can be seen, the tannin composition of cherry heartwood does not resemble even slightly that of oak used in cooperage, since in oak only higher concentrations of hydrolyzable tannins are found (*10*), even though toasting causes degradation in them as well. Other woods that can be used in cooperage, such as chestnut and acacia, also contain mainly hydrolyzable tannins, their levels being especially high in the case of chestnut.

A more detailed look at the phenolic composition of cherry wood was obtained through HPLC analysis, as can be seen in **Table 4**. As expected, toasting results in lignin degradation, leading to the formation of nonflavonoid phenolic compounds such as benzoic and cinnamic acids, and especially aldehydes, with the levels of 4-hydroxy-3,4-dimethoxy aldehydes being higher than those of 4-hydroxy-3-methoxy or 3,4-dihydroxy species. The formation of protocatechuic acid and aldehyde during toasting can be explained because the degradation of quercetin by heat, which produces the acid as a cleavage reaction product (*24*), and further heat reduction may result in the production of the aldehyde. Heat also causes the degradation of gallic and *p*-coumaric acids and 3,4,5-trimethoxyphenol but has no effect on methyl vanillate and syringate. In seasoned wood, protocatechualdehyde was the only aldehyde detected, probably because of the low levels of the other aldehydes (*8*) and the interference of flavonoid compounds in the HPLC analysis. Among all nonflavonoid compounds, vanillin is the most important from an organoleptic point of view when it comes to the aging of beverages, since it is an impact molecule with a vanilla smell. Its

Table 4. HPLC-DAD Quantitative Evaluation of Phenolic Compounds in Seasoned and Toasted Cherry Heartwood[a]

		(μg/g of wood)	
peak	compd	seasoned	toasted
	Nonflavonoid Compounds		
1	gallic acid	1.22	nd
2	protocatechuic acid	12.94	70.3
4	*p*-hydroxybenzoic acid	1.64	21.2
9	vanillic acid	2.04	9.90
15	syringic acid	nd	79.9
21	benzoic acid	10.9	19.8
22	*p*-coumaric acid	26.3	nd
3	protocatechualdehyde	7.59	78.4
13	vanillin	nd	41.9
19	syringaldehyde	nd	289
26	coniferaldehyde	nd	215
28	sinapaldehyde	nd	1637
29	methyl vanillate	9.82	9.92
31	methyl syringate	164	165
23	scopoletin	2.42	18.8
17	3,4,5-trimethoxyphenol	80.3	nd
	$\sum$ nonflavonoid	*315*	*2658*
	Procyanidins		
8	(+)-catechin	30150	151
18	(-)-epicatechin	362	nd
6	procyanidin B1	149	nd
12	procyanidin B2	717	nd
5	B-type procyanidin dimer	822	nd
10	B-type procyanidin dimer	227	nd
14	B-type procyanidin dimer	376	nd
16	B-type procyanidin dimer	289	nd
20	B-type procyanidin dimer	1718	nd
25	B-type procyanidin dimer	220	nd
7	B-type procyanidin trimer	1122	nd
11	B-type procyanidin trimer	131	nd
	$\sum$ Procyanidins	*36290*	*151*
	Other Flavonoids		
24	taxifolin	3581	102
27	tetrahydroxyflavanonol	190	nd
30	aromadendrin	4535	161
32	tetrahydroxyflavanone	113	nd
34	eriodictyol	1152	175
35	naringenin	7514	829
37	hesperetin	102	nd
38	trihydroxyflavanone	127	nd
41	pinocembrin	452	nd
42	isosakuranetin	3653	242
36	quercetin	801	342
39	kaempferol	416	85.9
43	kaempferide	35.5	nd
40	apigenin	76.6	27.9
33	tetrahydroxychalcone	20.1	nd
	$\sum$ other flavonoids	*22768*	*1965*

[a] Unidentified flavonoids were quantified as aromadendrin (peak 27), naringenin (peaks 32 and 38), and butein (peak 33). Unidentified procyanidins were quantified as procyanidin B2. nd = not detected.

concentration, in both seasoned and toasted cherry wood, was rather lower than in other woods used in cooperage, but inside the range of concentrations that can be expected for this compound (*10*, *11*). Moreover, the hydroxybenzoic and hydroxycinnamic acids that we found in cherry wood were recently related to an astringent mouth feel produced by red wine, and their ethyl esters also contribute to astringency, at low taste thresholds (*25*). Their concentrations were intermediate between those detected in European and American toasted oakwood (*10*); thus, we would expect a similar contribution to this sensation. Only the presence of protocatechuic acid, not detected in oak, could have an additive effect, but the low levels of gallic acid would compensate for this effect thoroughly. On the other hand, on comparison of the low-molecular-weight phenolic composition with that of oak wood, seasoned and toasted cherry wood shows some more differences: the presence of protocatechuic acid and aldehyde, *p*-coumaric acid, methyl vanillate, methyl syringate, 3,4,5-trimethoxyphenol, and benzoic acid, a very low amount of gallic acid, and the absence of ellagic acid. The presence of benzoic acid at appreciable levels may explain the higher levels of ethyl benzoate found in vinegars obtained by acetification in barrels of cherry wood than in those acetified in barrels made of other woods (*4*).

In seasoned wood we found a great variety of flavonoid compounds (**Table 4**) with concentrations ranging between 20 and more than 30 000 μg/g of wood, mainly the flavan-3-ols (+)-catechin, a B-type procyanidin dimer, and a B-type procyanidin trimer, the flavanones naringenin, isosakuranetin, and eriodictyol, and the flavanonols aromadendrin and taxifolin, all with concentrations higher than 1000 μg/g of wood. The concentrations of all these flavonoid compounds decreased after toasting by more than 90%, with the flavan-3-ol family being predominant, since while in seasoned wood they were the main compounds, in toasted wood their quantification was not possible due to their low levels and the interference of other compounds in the HPLC analysis. This is especially the case for (+)-catechin, the main compound in seasoned wood, found in diethyl ether, ethyl acetate, and freeze-dried extracts but only detected in the ethyl acetate extract obtained from toasted wood, in very low concentrations. The heat-induced degradation of flavonoid compounds has frequently been previously described in the cases of both flavan-3-ol and flavonols. Thus, a decrease of levels of procyanidins produced by heat was detected in peanut skins after roasting (175 °C, 5 min), as they are the most heat-sensitive monomers (*26*). Moreover, thermal processes such as boiling, frying, and microwave cooking reduce the flavonol content of vegetables (*27*), under both oxidant and autoxidant conditions (*24*). In toasted wood only nine flavonoids were quantified, and their concentrations were low, between 27 and 830 μg/g, with naringenin, quercetin, and isosakuranetin being the most abundant.

The toasting conditions applied provoked an important modification in the phenolic composition of cherry wood. Thus, toasting causes the degradation of condensed tannins, as well as an important decrease in the other flavonoids, and the degradation of lignin with the resulting increase of lignin derivative compounds. In oak wood, a lighter toasting process results in only minor degradation of wood components, and the same would probably be true in cherry wood, culminating in a different ratio of flavonoid to nonflavonoid compounds. Taking oak wood as a reference, in the interaction between cherry wood and the different kinds of beverages (wines, spirits, vinegars, ciders, etc.), some aspects of its phenolic composition should be kept in mind. If both toasted and untoasted barrels are used, cherry wood will not provide hydrolyzable tannins as oak does and, therefore, the chemical reactions in which oak ellagitannins usually participate during aging (*28*) will not take place. In untoasted barrels the tannins provided will be condensed tannins, rather similar to those already found in wine; thus, the beverages will increase their flavan-3-ol concentrations, resulting in the possible formation of new compounds during aging (*29*), as well as an increase of their antioxidant capacity (*30*). This antioxidant capacity will also be increased by the other flavonoids contributed by untoasted cherry wood, most of which have never been detected in these beverages before, such as taxifolin, aromadendrin, eriodictyol, naringenin, hesperetin, pinocembrin, isosakuranetin, and apigenin, and therefore the implications in the chemical modifications that take place during beverage aging, as well as in their organoleptic characteristics, are not known. On the other hand, untoasted cherry wood contributed very low quantities of lignin derivatives in a way quite different from that of oak, since compounds such as protocatechuic acid and aldehyde, *p*-coumaric acid, methyl vanillate, methyl syringate, and benzoic acid are included, but not ellagic acid, and only a small amount of gallic acid. If toasted barrels are used, the cherry wood will not contribute tannins, and only a very few other flavonoids and lignin derivatives, in higher quantities than for untoasted but in lower concentrations than are normally found in medium toasted oak wood. Probably, different toasting levels of cherry wood would result in different ratios of flavonoid to nonflavonoid compounds, and a detailed study how toasting intensity affects the phenolic composition of cherry wood would be of interest for a more complete evaluation of the impact of this wood in the aging of different beverages, taking into account that the different beverages have different capacities for removing phenolic compounds from wood.

ACKNOWLEDGMENT

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

LITERATURE CITED

(1) Holdefer, M.; Fischer, U. Ageing of fruit brandies in wooden barrels: effect of various wood types. I. *Kleinbrennerei* **1997**, *49*, 231–233.

(2) Del Campo, G.; Santos, J. I.; Berregi, I.; Velasco, S.; Ibarburu, I.; Dueñas, M. T.; Irastorza, A. Ciders producers by two types of presses and fermented in stainless steel and wooden vats. *J. Inst. Brewing* **2003**, *109*, 342–348.

(3) Caldeira, I.; Climaco, M. C.; Sousa, B.; Belchior, A. P. Volatile composition of oak and chestnut woods used in brandy ageing: modification induced by heat treatment. *J. Food Eng.* **2006**, *76*, 202–211.

(4) Cerezo, A. B.; Tesfaye, W.; Torija, M. J.; Mateo, E.; García-Parrilla, C.; Troncoso, A. M. The phenolic composition of red wine vinegar produced in barrels made from different woods. *Food Chem.* **2008**, *109*, 606–615.

(5) De Rosso, M.; Panighel, A.; Dalla Vedova, A.; Stella, L.; Flamini, R. Changes in chemical composition of a red wine aged in acacia, cherry, chestnut, mulberry, and oak wood barrels. *J. Agric. Food Chem.* **2009**, *57*, 1915–1920.

(6) Torija, M. J.; Mateo, E.; Vegas, C. A.; Jara, C.; González, A.; Poblet, M.; Reguant, C.; Guillamon, J. M.; Mas, A. Effect of wood type and thickness on acetification kinetics in traditional vinegar production. *Int. J. Wine Res.* **2009**, *1*, 155–160.

(7) Flamini, R.; Dalla Vedova, A.; Cancian, D.; Panighel, A.; De Rosso, M. GC/MS-positive ion chemical ionization and MS/MS study of volatile benzene compounds in five different woods used in barrel making. *J. Mass Spectrom.* **2007**, *42*, 641–646.

(8) Fernández de Simón, B.; Esteruelas, E.; Muñoz, A. M.; Cadahía, E.; Sanz, M. Volatile compounds in acacia, chestnut, cherry, ash, and

oak woods, with a view to their use in cooperage. *J. Agric. Food Chem.* **2009**, *57*, 3217–3227.
(9) De Rosso, M.; Cancian, D.; Panighel, A.; Dalla Vedova, A.; Flamini, R. Chemical compounds released from five different woods used to make barrels for aging wines and spirits: volatile compounds and polyphenols. *Wood Sci. Technol.* **2009**, *43*, 375–385.
(10) Cadahía, E.; Fernández de Simón, B. *Utilización del roble español en el envejecimiento de vinos. Comparación con roble francés y americano*; INIA: Madrid, 2004; pp 1−136.
(11) Cadahía, E.; Fernández de Simón, B.; Poveda, P.; Sanz, M. *Utilización de Quercus pyrenaica Willd. de Castilla y León en el envejecimiento de vinos. Comparación con roble francés y americano*; INIA: Madrid, 2008; pp 1−130.
(12) Vinciguerra, V.; Luna, M.; Biston, A.; Zollo, F. Variation in the composition of the heartwood flavonoids of *Prunus avium* by on-column capillary gas chromatography. *Phytochem. Anal.* **2003**, *14*, 373–377.
(13) McNulty, J.; Nair, J. J.; Bollareddy, E.; Keskar, K.; Thorat, A.; Crankshaw, D. J.; Holloway, A. C.; Khan, G.; Wright, G. D.; Ejim, L. Isolation of flavonoids from the heartwood and resin of *Prunus avium* and some preliminary biological investigations. *Phytochemistry* **2009**, *70*, 2040–2046.
(14) Singleton, V. L.; Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic and phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144.
(15) Swain, T.; Hillis, W. E. The phenolic constituents of *Prunus domestica*. I. The quantitative analysis of phenolic compounds. *J. Sci. Food Agric.* **1959**, *10*, 63.
(16) Cadahía, E.; Conde, E.; Fernández de Simón, B.; García-Vallejo, M. C. Changes in tannic composition of reproduction cork Quercus suber throughout the industrial processing. *J. Agric. Food Chem.* **1998**, *46*, 2332–2336.
(17) Porter, L. J.; Hirstich, L. N.; Chan, B. G. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry* **1986**, *25*, 223.
(18) Ma, Y. L.; Li, Q. M.; Van den Heuvel, H.; Claeys, M. Characterization of flavone and flavonol aglycones by CID MS-MS. *Rapid Commun. Mass Spectrom.* **1997**, *11*, 1357.
(19) Porter, L. J. Flavans and proanthocyanidins. In *The Flavonoids: Advances in Research since 1980*; Harborne, J. B., Ed.; Chapman and Hall: London, 1980; pp 21−62.
(20) Dueñas, M.; Sun, B.; Hernández, T.; Estrella, I.; Spranger, M. I. Proanthocyanidin composition in the seed coat of lentils (*Lens culinaris* L.). *J. Agric. Food Chem.* **2003**, *51*, 7999–8004.
(21) Tretter, D.; Galensa, R.; Feucht, W.; Schmid, P. P. Flavanone glucosides in callus and phloem of *Prunus avium*: Identification and stimulation of their synthesis. *Physiol. Plant.* **1985**, *65*, 95–101.
(22) Fabre, N.; Rustan, I.; Hoffmann, E.; Quetin-Leclercq, J. Determination of flavone, flavonol, and flavanone aglycones by negative ion liquid chromatography electrospray ion trap mass spectrometry. *J. Am. Soc. Mass Spectrom.* **2001**, *12*, 707–715.
(23) Bohm, B. *Introduction to Flavonoids*; Harwood Academic: Amsterdam, The Netherlands, 1998, pp 1−503.
(24) Buchner, N.; Krumbein, A.; Rohn, S.; Kroh, L. H. Effect of thermal processing on the flavonols rutin and quercetin. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 3229–3235.
(25) Hufnagel, J. C.; Hofmann, T. Orosensory-directed identification of astringent mouthfeel and bitter-tasting compounds in red wine. *J. Agric. Food Chem.* **2008**, *56*, 1376–1386.
(26) Yu, J.; Ahmedma, M.; Goktepe, I.; Dai, J. Peanut skin procyanidins: composition and antioxidant activities as affected by processing. *J. Food Comp. Anal.* **2006**, *19*, 364–371.
(27) Crozier, A.; Lean, M. E. J.; Mc Donald, M. S.; Black, C. Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce, and celery. *J. Agric. Food Chem.* **1997**, *45*, 590–595.
(28) Fujieda, M.; Tanaka, T.; Suwa, Y.; Koshimizu, S.; Kouno, I. Isolation and structure of whiskey polyphenols produced by oxidation of oak wood ellagitannins. *J. Agric. Food Chem.* **2008**, *56*, 7305–7310.
(29) Es-Safi, N. E.; Cheynier, V. Flavanols and anthocyanins as potent compounds in the formation of new pigments during storage and aging of red wine. In *Red Wine Color-Revealing the Mysteries*; Waterhouse, A. L., Kennedy, J. A., Eds.; American Chemical Society: Washington, DC, 2004; ACS Symposium Series 886, pp 143−159.
(30) Shahidi, F.; Wanasundara, P. K. Phenolic antioxidants. *Crit. Rev. Food Sci. Nutr.* **1992**, *32*, 67.

Received for review January 20, 2010. Revised manuscript received March 10, 2010. Accepted March 11, 2010.