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Changes in Chemical Composition of a Red Wine Aged in Acacia, Cherry, Chestnut, Mulberry, and Oak Wood Barrels

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Aging in wooden barrels is a process used to stabilize the color and enrich the sensorial characteristics of wine. Many compounds are released from wood into the wine; oxygen permeation through the wood favors formation of new anthocyanin and tannin derivatives. Recently, polyphenols and volatile compounds released from acacia, chestnut, cherry, mulberry, and oak wood used in making barrels for spirits and wine aging were studied. Here, changes in volatile and polyphenolic compositions of a red wine aged for 9 months in acacia, cherry, chestnut, mulberry, and oak barrels are studied. Mulberry showed significant decreases of fruity-note ethyl esters and ethylguaiacol and a great cession of ethylphenol (horsey-odor defect). Cherry promoted the highest polyphenol oxidation, making it less suitable for long aging. LC/ESI-MS^n showed the relevant presence of *cis*- and *trans*-piceatannol in mulberry-aged wine, a phytoalexin with antileukemia and antimelanoma activities.

KEYWORDS: wine; wood barrels; aroma; polyphenols; SPME; LC/MS

INTRODUCTION

Aging of wines and spirits in wooden barrels is a process used to stabilize color, improve limpidity, and enrich the sensorial characteristics of the product. Many types of compounds are transferred from the wood to the wine, such as polyphenols, lactones, coumarins, polysaccharides, hydrocarbons and fatty acids, terpenes, norisoprenoids, steroids, carotenoids, and furan compounds. Volatile phenols and benzoic aldehydes are particularly significant, as they confer important sensorial characteristics to products (*1*−*5*). Compounds such as benzaldehyde and derivatives, vanillin and syringaldehyde, cinnamaldehyde, coniferaldehyde and sinapinaldehyde, eugenol and methoxyeugenol, guaiacol, vinylguaiacol and derivatives, β-methyl-γ-octalactones, furfural, and 5-methylfurfural and derivatives contribute toward forming aromas in aged wine. Vanillin (vanilla note) and eugenol (clove, spicy note) are characterized by typical sensorial properties and low sensory thresholds in wine (0.3 and 0.5 ppm, respectively) (*6*); furan and pyran derivatives form as a consequence of heating wood during barrelmaking and are characterized by a toasty caramel aroma (*7*).

Permeation of oxygen through barrel staves is due to wood porosity (for oak barriques, it has been estimated at 10−45 mg/L per year) and promotes formation of new stable anthocyanin and tannin derivatives, with consequent color stabilization and loss of astringency of wines (*8*).

The main polyphenols from wood are gallotannins and ellagitannins (hydrolyzable tannins), which play a very important role in wine affinity (*2, 8*). Oxygen reacts with hydrolyzable tannins, forming H_2O_2 and inducing oxidation of ethanol into ethanal, which reacts with wine tannins to form tannin−ethanal intermediates. These compounds combine with anthocyanins to form new stable pigments (*8, 9*).

Oak (*Quercus sessilis*, *Q. petraea*, *Q. robur*, *Q. pedunculata*, *Q. alba*) is the wood commonly used in making barrels for wine aging (*10*). In the past, chestnut (*Castanea sativa*) was widely used for enological purposes in the Mediterranean area, due to its wide diffusion and low cost.

Recently, polyphenols and volatile compounds released from wood of acacia (*Robinia pseudoacacia*), chestnut, cherry (*Prunus avium*), mulberry (*Morus alba* and *M. nigra*), and oak in a model spirit (50% ethanol) and a model wine (tartrate buffer, pH 3.2−12% ethanol) solution were studied, and volatile benzene compounds were identified by ion trap collision-induced dissociation (CID) and tandem mass spectrometry (MS/MS) experiments on the protonated $[M + H]^+$ species produced in positive ion chemical ionization (PICI) (*11, 12*). These woods showed very different chemical proprieties: acacia is characterized by significant contents of benzene aldehydes, chestnut by richness in polyphenols and relevant cession of eugenol and vanillin to the wine, cherry by release of methoxyphenols, mulberry by the lowest

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Table 1. Mean Data and Maximum Error of the Principal Fermentative, Wood-Released, and Grape Aroma Compounds Determined by Two Repeated SPME-GC/MS Analyses of Raboso Piave Wine Samples Aged in Acacia, Cherry, Chestnut, Mulberry, and Oak 225-L Wood Barrels

		compounds (mg/L)								
barrel	months of aging	isoamyl acetate[a]	ethyl hexanoate[a]	ethyl octanoate[a]	4-ethylphenol[a]	4-ethylguaiacol[b]	furfural[a]	5-methylfurfural[b]	eugenol[a]	vanillin[a]
acacia	3	3.37 ± 0.80	1.99 ± 0.23	14.78 ± 2.81	0.67 ± 0.07	2.24 ± 0.21	0.02 ± 0.01	0.03 ± 0.01	0.009 ± 0.001	0.09 ± 0.03
	6	3.67 ± 0.35	1.91 ± 0.23	11.17 ± 1.23	0.92 ± 0.08	2.94 ± 0.14	0.04 ± 0.01	0.03 ± 0.01	0.015 ± 0.001	0.16 ± 0.01
	9	3.57 ± 0.07	1.56 ± 0.15	8.95 ± 1.73	1.29 ± 0.41	3.25 ± 0.67	0.03 ± 0.01	0.03 ± 0.01	0.021 ± 0.005	0.31 ± 0.07
cherry	3	4.14 ± 0.32	1.57 ± 0.25	10.22 ± 0.12	1.00 ± 0.44	3.01 ± 0.13	nd[c]	nd	0.008 ± 0.004	0.08 ± 0.04
	6	6.81 ± 1.68	1.82 ± 0.04	8.64 ± 0.14	1.04 ± 0.06	3.13 ± 0.26	tr[c]	nd	0.009 ± 0.001	0.10 ± 0.01
	9	4.13 ± 0.01	1.62 ± 0.32	7.57 ± 1.47	0.86 ± 0.18	2.79 ± 0.51	nd	nd	0.007 ± 0.001	0.12 ± 0.03
chestnut	3	3.36 ± 0.65	1.73 ± 0.17	11.91 ± 0.57	0.84 ± 0.20	2.53 ± 0.43	0.04 ± 0.02	0.03 ± 0.01	0.024 ± 0.004	0.45 ± 0.06
	6	3.51 ± 0.70	2.18 ± 0.34	10.53 ± 0.60	0.74 ± 0.08	2.30 ± 0.12	0.04 ± 0.01	0.02 ± 0.02	0.035 ± 0.003	0.60 ± 0.02
	9	3.25 ± 0.19	1.47 ± 0.18	9.14 ± 0.56	0.64 ± 0.04	1.84 ± 0.18	0.07 ± 0.01	0.04 ± 0.01	0.026 ± 0.002	0.43 ± 0.03
mulberry	3	5.85 ± 0.58	2.45 ± 0.69	14.19 ± 0.60	1.06 ± 0.26	2.69 ± 0.75	tr	nd	0.004 ± 0.001	0.09 ± 0.03
	6	2.94 ± 0.56	1.54 ± 0.05	12.06 ± 2.15	1.27 ± 0.26	2.72 ± 0.44	tr	nd	0.006 ± 0.001	0.08 ± 0.02
	9	4.21 ± 0.31	1.66 ± 0.28	10.31 ± 2.79	1.19 ± 0.07	1.84 ± 0.20	tr	tr	0.006 ± 0.001	0.08 ± 0.01
oak	3	5.27 ± 1.27	1.89 ± 0.47	11.15 ± 2.67	0.90 ± 0.07	2.51 ± 0.14	0.18 ± 0.08	0.14 ± 0.04	0.009 ± 0.001	0.27 ± 0.04
	6	3.43 ± 0.39	1.40 ± 0.07	9.60 ± 0.23	0.75 ± 0.05	2.08 ± 0.02	0.56 ± 0.16	0.19 ± 0.05	0.012 ± 0.003	0.34 ± 0.08
	9	2.09 ± 0.03	1.45 ± 0.21	7.61 ± 0.14	1.06 ± 0.36	2.90 ± 0.75	0.60 ± 0.06	0.32 ± 0.04	0.018 ± 0.005	0.36 ± 0.09

[a] Compound identified by commercial references. [b] Tentative identification based on NIST98 library mass spectra and GC retention times reported in literature. [c] nd, not detected; tr, trace (<0.01 ppm).

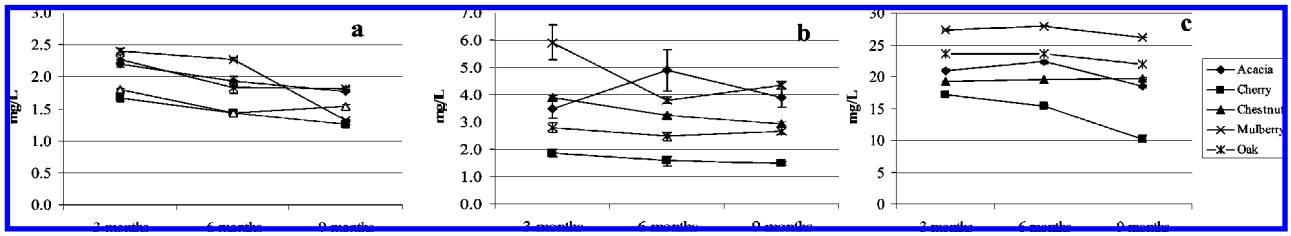

Figure 1. Trends of myricetin glucoside (**a**), quercetin glucoside (**b**), and (+)-catechin (**c**) in Raboso Piave wine during aging. Bars: variability of duplicate analyses.

volatiles, and oak by β-methyl-γ-octalactones and polyphenols stable to oxidation.

To our knowledge, no studies on the chemical changes of wines aged in acacia, cherry, chestnut, and mulberry barrels have been reported in the literature. Due to increasing interest in the use of these woods for the production of typical wines, in particular in Italy, in this study the suitability of these barrels for enological purposes was evaluated. For this aim, the chemical composition of a red wine aged in 225-L barrels (barriques) was studied for a period of 9 months, and samples were compared with the oak-aged wine. Contents of volatile compounds from grape, formed with fermentation and from wood, and of wine polyphenols such as hydroxycinnamoyltartaric acids, flavonols, (+)-catechin, and anthocyanins were monitored. The compounds corresponding to new signals observed in the LC chromatogram of cherry- and mulberry-aged wines were studied by negative-ion LC/ESI-MS^n.

MATERIALS AND METHODS

Samples. For our purposes, five barriques (225-L barrels) of acacia (*R. pseudoacacia*), chestnut (*C. sativa*), cherry (*P. avium*), mulberry (*M. alba*), and oak (*Q. petrea*) were made from Veneta Botti srl (Veneto, Italy). Untoasted woods, 24−36 months naturally seasoned, and an oak wood sample subjected to a light toasting treatment (40 min of heating by fire for bending of staves) were used. Each barrel was 80% filled with a red wine (Raboso Piave) produced in 2005 with an ethanol content of 12.5% (v/v) and a total acidity of 9.5 g/L expressed as tartaric acid. Wine samples were aged at cellar temperature (15−18 °C) for a period of 9 months. Two samples of each wine were collected after 3, 6, and 9 months and analyzed. The mean data of each parameter were calculated.

Table 2. Total Flavonoids and Anthocyanins of Raboso Piave Wine Samples during Aging

barrel	months of aging	total flavonoids, (+)-catechin (mg/L)	total anthocyanins, malvidin-3-*O*-glucoside (mg/L)
acacia	3	1900	216
	6	1845	194
	9	1929	204
cherry	3	1477	169
	6	1390	144
	9	1398	139
chestnut	3	1635	220
	6	1799	213
	9	1756	215
mulberry	3	1904	233
	6	1914	214
	9	1906	240
oak	3	1836	242
	6	1722	210
	9	1730	218

Solvents and Standards. HPLC grade methanol was purchased from Baker (Phillipsburg, NJ) and superpurity formic acid from Romil Ltd. (Cambridge, U.K.). Standards of isoamyl acetate, guaiacol, 1-heptanol, and furfural were purchased from Carlo Erba Reagents (Milan, Italy);

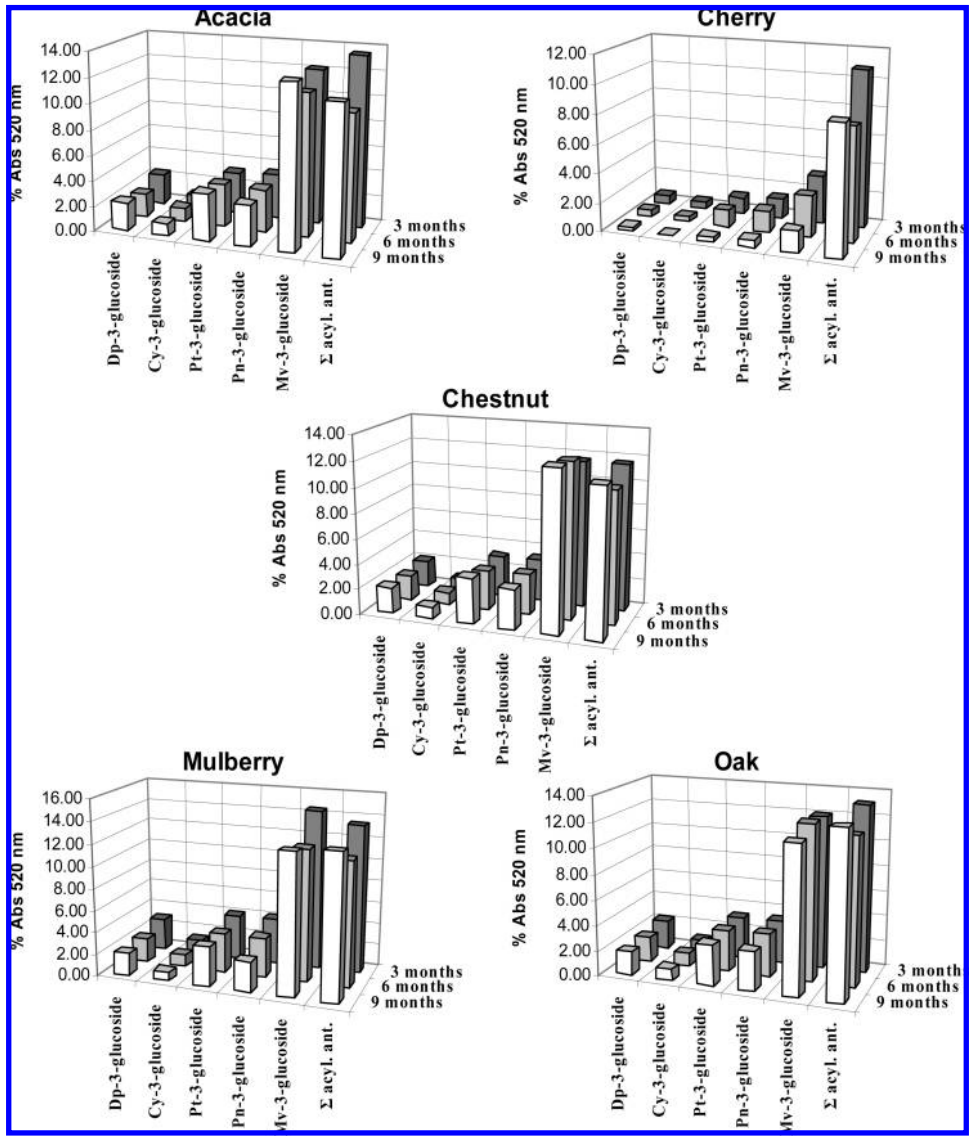

Figure 2. Anthocyanins in Raboso Piave wine samples in acacia, cherry, chestnut, mulberry, and oak wood barrels. Each compound is expressed as peak area percentage of the sum of peak areas in LC/UV chromatogram at 520 nm. Σacyl.ant, sum of acetate and *p*-coumarate anthocyanins.

eugenol, 4-ethylphenol, *trans*-ferulic acid, *trans*-caffeic acid, *trans-p*-coumaric acid, and (+)-catechin were from Sigma-Aldrich (Milan, Italy); vanillin, myricetin, and quercetin glucoside were from Fluka (Buchs, Switzerland); kaempferol glucoside was from Extrasynthese (Genay, France); ethyl hexanoate and α-terpineol were from BDH Chemicals Ltd. (Poole, U.K.); ethyl octanoate was from Eastman Organic Chemicals (Rochester, NY); and citronellol was purchased from Merck KGaA (Darmstadt, Germany).

Aroma Compounds Analysis. Volatile compounds were determined by headspace (HS) solid phase microextraction−gas chromatography−mass spectrometry (SPME-GC/MS) using the method according to Carrillo et al. (*13*) modified for our purposes. A volume of 10 mL of wine was transferred in a 20-mL sealed vial, and 3 g of NaCl and 100 μL of a 1-heptanol 180 mg/L solution (internal standard) were added. Before extraction, the sample was incubated at 70 °C for 10 min, and then extraction was performed by a carbowax/divinylbenzene (CAR/DVB) 65-μm fiber (Supelco, Bellafonte, PA) for 30 min at 70 °C under stirring at 300 rpm. Before the sampling, the SPME fiber was done by thermal conditioning in accordance with the manufacturer's recommendations. Analyses were performed by an HP 5890 gas chromatograph coupled with an HP 5971 mass spectrometer (Agilent Technologies, Santa Clara, CA) equipped with an HP Innowax fused silica capillary column (30 m × 0.25 mm i.d.; df 0.25 μm) (Agilent Technologies). Thermal desorption of analytes from the fiber into the GC injection port was performed at 230 °C for 5 min in splitless mode. Conditions: carrier gas, He; column head pressure, 12 psi constant pressure; oven temperature program, 5 min at 40 °C, raised at 3 °C/min to 230 °C, 10 min at 230 °C. The mass spectrometer operated in singular ion monitoring (SIM) mode with an electron energy of 70 eV and a solvent delay of 3.5 min. Calibration curves were calculated using model wine solutions (tartaric buffer pH 3.2 and 12% ethanol) containing the analytes in the concentration ranges of 20−0.01 ppm and 2−0.001 ppm for eugenol: isoamyl acetate (mg/L) = 4E + 06*x* (*m*/*z* 70, $R^2 = 0.999$); ethyl hexanoate (mg/L) = 7E + 06*x* (*m*/*z* 88, $R^2 = 1$); ethyl octanoate (mg/L) = 5E + 06*x* (*m*/*z* 88, $R^2 = 0.999$); furfural (mg/L) = 3.7E + 07*x* (*m*/*z* 96, $R^2 = 0.999$), α-terpineol (mg/L) = 5E + 07*x* (*m*/*z* 93, $R^2 = 0.999$); citronellol (mg/L) = 5E + 07*x* (*m*/*z* 69, $R^2 = 0.999$); guaiacol (mg/L) = 1E + 07*x* (*m*/*z* 124, $R^2 = 1$); eugenol (mg/L) = 5E + 07*x* (*m*/*z* 164, $R^2 = 1$); 4-ethylphenol (mg/L) = 1E + 08*x* (*m*/*z* 107, $R^2 = 1$); vanillin (mg/L) = 1E + 06*x* (*m*/*z* 152, $R^2 = 0.999$). Other signals recorded were *m*/*z* 70 for 1-heptanol, *m*/*z* 110 for 5-methylfurfural, and *m*/*z* 152 for 4-ethylguaiacol. 5-Methylfurfural and 4-ethylguaiacol were quantified on the furfural and 4-ethylphenol calibration curve, respectively. For each sample two analyses were performed, and the mean data of each parameter were calculated.

Polyphenols. *LC/DAD Analyses.* A ThermoFinnigan-Spectra System (Thermo, San Jose, CA), equipped with an SCM100 degasser, a P4000 pump, an AS3000 autosampler, and a UV6000LP DAD detector, was used. For analysis of hydroxycinnamic and hydroxycinnamoyltartaric acids, flavonols, and (+)-catechin the methods previously

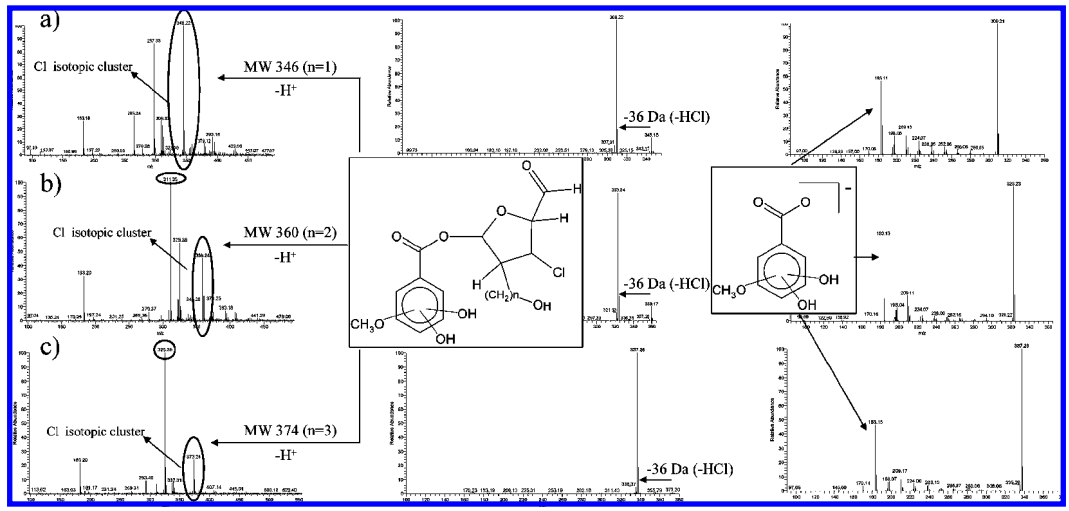

Figure 3. Direct ESI(−) full-scan mass spectra and MS/MS and MS^3 of $[M - H]^-$ ions corresponding to peaks at 51.51, 52.08, and 52.61 min in the TIC chromatogram of cherry-aged wine. Structures are proposed on the basis of fragmentation patterns.

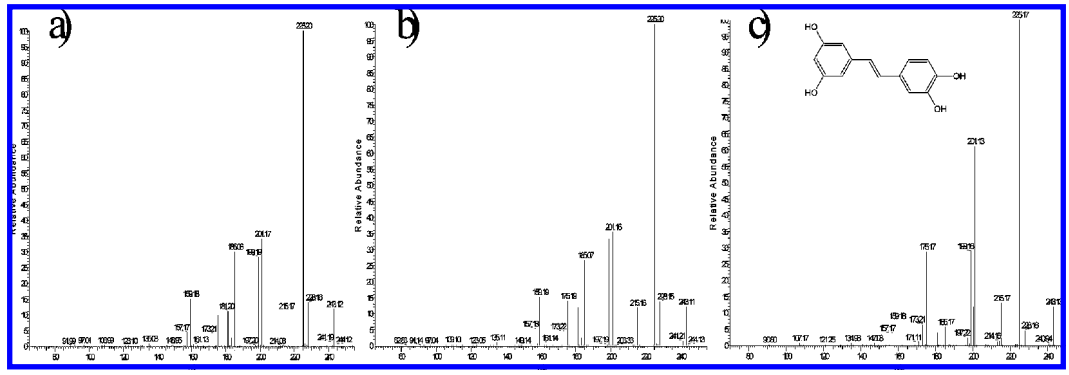

Figure 4. ESI-MS/MS fragmentation spectra of species at *m/z* 243, corresponding to peaks at 18.33 min (**a**) and 26.96 min (**b**) in the TIC chromatogram of mulberry-aged wine, and that of *trans*-piceatannol standard (**c**).

proposed were used (*14*). A volume of 4.5 mL of wine was acidified with 0.5 mL of 1 M H_3PO_4 and filtered with a 0.2-μm GHP membrane Acrodisc filter (Waters Corp., Milford, MA). Separation was carried out by a Hypersil-Keystone ODS Hypersil RP C18 column (200 × 2.1 mm, 10 μm) (Thermo) at room temperature. Eluent A was H_3PO_4 10^{-3} M, and eluent B was CH_3OH. The elution gradient program was as follows: from 5 to 10% of B in 5 min, from 10 to 30% B in 15 min, from 30 to 40% B in 20 min, from 40 to 100% B in 10 min, 100% B for 5 min, from 100 to 5% B in 5 min, conditioning for 15 min; flow rate, 250 μL/min. Sample injection volume was 20 μL. The chromatogram of hydroxycinnamoyltartaric acids (HCTA) was recorded at 320 nm, that of flavonols at 360 nm, and that of (+)-catechin at 280 nm.

For analysis of anthocyanins, 1 mL of wine was passed through a 360-mg C_{18} Sep-Pak cartridge (Waters Corp., Milford, MA) previously activated with 3 mL of methanol and 5 mL of water. After sample passage, the cartridge was washed with 2 mL of 0.01 M H_2SO_4, and anthocyanis were eluted with 3 mL of CH_3OH/HCl 0.1% (v/v) solution and filtered with a 0.2-μm GHP Acrodisc filter. The anthocyanin profile was determined using a LiChrospher 100 RP18 column (250 × 4.6 mm; 5 μm) (Merck KGaA) at 30 °C. Eluent A was H_2O/HCOOH 90:10 (v/v), and eluent B was H_2O/HCOOH/CH_3OH 40:10:50 (v/v/v). The elution gradient program was as follows: from 10 to 45% of B in 20 min, from 45 to 70% B in 25 min, from 70 to 90% B in 10 min, from 90 to 99% B in 2 min, 99% B for 10 min, from 99 to 10% B in 5 min, conditioning for 8 min; flow rate, 0.7 mL/min; sample injection volume, 20 μL; detection wavelength, 520 nm (*14*). Two analyses of each sample were performed, and the mean data of each parameter were calculated.

LC/ESI-MS^n Analysis. The mass spectrometer was an LCQDeca ion trap instrument (Thermo) operating in electrospray ionization (ESI) conditions coupled with an Ultimate 3000 HPLC System (Dionex Corp., Sunnyvale, CA). A Spectra System UV 1000 detector (Thermo) was connected to the HPLC system.

Separation was performed by a LiChrospher 100 RP18 column (250 × 4.6 mm; 5 μm). Eluent A was water/0.1% HCOOH, and eluent B was CH_3OH. The elution gradient program was as follows: 33% of B for 40 min, from 33 to 100% B in 15 min, 100% B for 5 min, from 100 to 33% B in 1 min; flow rate, 600 μL/min. Sample injection volume was 20 μL with a split ratio of 1:1 for simultaneous MS and spectrophotometric detection at 320 nm.

ESI parameters were as follows: source voltage, 4500 V; entrance capillary voltage, 4 V; entrance capillary temperature, 280 °C; sheath gas flow rate, 60 (arbitrary units); auxiliary gas flow rate, 20 (arbitrary units). The significant data were recorded in negative ion mode. The scan range was fixed from *m/z* 70 to 700. Structural identification of the ions produced by ESI was performed by means of multiple mass spectrometry experiments and was done by applying supplementary radiofrequency voltage in the range of 2 V to the end-caps of the ion trap in order to make selected ions collide with helium present in the ion trap as buffer gas at a pressure of 1.1×10^{-5} Torr.

Spectrophotometric Analyses. All analyses were performed by a Uvikon 930 UV−vis spectrophotometer (Kontron Instruments, Milan, Italy). Indices of total flavonoids and anthocyanins were determined according to the method proposed by Di Stefano et al. (*15*). Wine samples were diluted 1:25 v/v (for anthocyanins) and 1:50 v/v (for flavonoids) with a CH_3CH_2OH/H_2O/HCl 70:30:1 (v/v/v) solution, and the 230−700 nm absorbance spectrum was recorded. The index of total flavonoids was calculated as (+)-catechin (mg/L) = $Abs_{280corr} \times 82.4 \times 50$, with $Abs_{280corr}$ the maximum of absorbance recorded at 280 nm corrected by the nonphenolic compound noise. The index of total anthocyanins was calculated as malvidin-3-*O*-monoglucoside (mg/L)

$= Abs_{max540} \times 16.17 \times 25$. All analyses were repeated in duplicate, and the mean data were calculated.

RESULTS AND DISCUSSION

Volatile Compounds. Volatile compounds determined by SPME-GC/MS are listed in **Table 1**. These analytes were selected because they are the principal fermentative compounds (more abundant fruity-note esters and negative phenols), aromas released from wood (vanillin, eugenol, furfurals from toasting), and grape variety aromas (some terpenols for Raboso Piave). As evidenced from the calibration curves, the CAR/DVB fiber provides satisfying recoveries and linearity for all compounds.

As acacia, cherry, chestnut, and mulberry barrels were made with untoasted woods, significant furfural and 5-methylfufural were found only in oak-aged wine. Guaiacol was not found at a detectable level in any samples.

The Raboso Piave wine sample studied showed high ethyl octanoate content, up to 10-fold that normally reported in wines. In general, decreases between 20 and 40% of fermentative compounds characterized by fruity fragrances, in particular ethyl octanoate, were observed in the last 6 months. The highest decrease of isoamyl acetate after 9 months of aging was found in the oak-aged sample.

As found in model solutions wood extract, also in wine, acacia, chestnut, and oak showed substantial cession of vanillin and eugenol (*11*). Wine aged in acacia showed a constant increase in ethylguaiacol (spicy note), and at the end of aging this compound was highest in this sample. Mulberry-aged wine was characterized by a significant decrease in fruity-note ethyl esters and ethylguaiacol and a high amount of ethylphenol (1.0−1.3 mg/L), a horsey-odor wine defect. Cherry-aged wine had high ethylguaiacol already after 3 months of aging. In this sample, a significant increase of isoamyl acetate was observed after 6 months with a concomitant increase of acetic acid (data not shown), suggesting a higher oxygen permeation through the staves compared to the other barrels.

In general, no significant differences in grape aromas (α-terpineol and citronellol) among the various samples (data not shown) were found.

Polyphenols. The study was performed on a Raboso wine, because this variety is characterized by a richness of polyphenols and a high anthocyanin content. Due to oxygen permeation through the wood, which favors the formation of new derivatives, the study focused on polyphenols with higher oxidizability.

In general, during aging, no significant changes in hydroxycinnamoyltartaric acids (in particular, *cis*- and *trans*-caffeyltartaric and caffeic acids) were observed (data not shown). **Figure 1**, panels **a**, **b**, and **c**, shows trends of myricetin glucoside and quercetin glucoside (the principal flavonols of Raboso wine) and (+)-catechin, respectively. A great decrease in flavonols was observed: after 3 months of aging, the lowest values were found in the cherry-aged sample and, after 9 months, myricetin glucoside and quercetin glucoside decreased by 30 and 44% with respect to oak-aged wine, respectively. After 9 months, higher quercetin glucoside was found in acacia (+46% with respect to oak) and mulberry aged (+63%) wines.

(+)-Catechin was lowest in the cherry-aged sample, with a marked decrease in the last 3 months of aging. After 9 months, higher levels were found in mulberry, oak, and acacia wines.

Anthocyanins and total flavonoids were not significantly influenced by barrel type, except for the cherry-aged sample, for which, again, an important decrease in polyphenol contents after the first 3 months was already observed (**Table 2**). Unlike all of the other samples, cherry-aged wine also revealed profound changes in the anthocyanin profile, due to rearrangements, polymerization, and oxidation reactions of anthocyanins (**Figure 2**).

LC/ESI-MS^n Characterization of New Peaks in LC/UV Chromatograms. The LC/UV chromatogram at 320 nm of cherry-aged wine was also different from the other samples also due to the presence of a new peak cluster in the 50−54-min range. A tentative characterization of these compounds was performed by negative-ion LC/ESI-MS^n. The total ion current (TIC) chromatogram showed three main peaks at 51.51, 52.08, and 52.61 min. ESI(−) full-scan mass spectra of all these peaks had an isotopic cluster characteristic of molecules containing one chlorine atom, and molecular masses of compounds differed by +14 and +28 Da, respectively. Tandem mass spectrometry MS/MS and MS^3 experiments on the $[M - H]^-$ ion showed a 36-Da loss (corresponding to a HCl molecule) for all compounds, with formation of fragments at *m*/*z* 309, 323, and 337. These fragments showed a successive neutral loss of 126, 140, and 154 Da, respectively (perhaps corresponding to alkylhydroxylated furfural-like structures typical of lignins), all fragmentations leading to the formation of a signal at *m*/*z* 183, probably corresponding to a methoxygallate, and a final CO_2 loss (**Figure 3**). These chlorinated compounds were probably due to lignin degradation as a consequence of wood sanitization treatments (with chlorine or other chlorinated products) during barrelmaking.

The LC/UV chromatogram at 320 nm of mulberry-aged wine showed an intense peak at 26.44 min. The ESI(−) full-scan mass spectrum of this signal showed as base peak, probably corresponding to $[M - H]^-$ ion, the signal at *m*/*z* 243. The same was observed for a less intense peak in the chromatogram at 18.34 min. MS/MS experiments on the *m*/*z* 243 species of two peaks led to identification of *cis*- and *trans*-piceatannol, confirmed by LC and MS/MS analyses of standard *trans*-piceatannol (**Figure 4**). The content of piceatannol found in the wine was several tens of milligrams per liter.

In conclusion, even though the study was focused on a specific wine, useful results potentially applicable to red wine aging were achieved: the significant decrease of fruity-note ethyl esters and ethylguaiacol and the high cession of ethylphenol found in mulberry-aged wine indicate that this wood is less suitable for wine aging; in general, the lowest content of oxidizable polyphenols found in cherry-aged wine indicates this type of wood is the most oxidative environment, and it is less suitable for long aging followed by chestnut; oak, mulberry, and acacia barrels resulted in the least oxidative environments.

The content of piceatannol found in the mulberry-aged wine is interesting because this compound is a hydroxystilbene phytoalexin with antileukemia and antimelanoma activities, which has been reported to be of interest in the production of anticancer and anti-Epstein−Barr virus drugs (*16*−*18*).

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