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Changes in phenolic composition of red wines aged in cherry wood

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ABSTRACT

The evolution of low molecular weight phenolic compounds in red wines aged in cherry (*Prunus avium*) or oak (*Quercus petrae*) wood has been investigated. In addition, the phenolic composition of hydro-alcoholic extracts of cherry heartwood has been characterized and quantified by means of HPLC-DAD/MS analysis.

More than 20 phenolic compounds, constitutive of cherry wood, were identified, including flavanols, flavanones, flavanonols, flavonols and flavones. During ageing, some of these compounds (eryodictiol, sakuranetin, pinocembrin and chrysin) were transferred to the wines and may represent putative phenolic markers of the usage of this wood specie.

The phenolic composition of wines was significantly affected by the different woods, the cherry barriques promoting the fastest evolution of (+)-catechin, procyanidins and flavonols if compared to oak.

Our findings confirmed that cherry wood is highly oxidative towards wine phenolics but, at the same time, suggested that a portion of those phenols are involved in condensation phenomena able to stabilize both the tannins and the pigments of the aged red wines.

1. Introduction

Wood ageing is a well established practice in the production of high quality red wines. This technique can promote the migration from the wood into the wine of a number of compounds which may positively influence the complexity and intensity of flavor and aroma (Jarauta, Cacho, & Ferreira, 2005). In addition, because of both the porosity of the wood fibers and the presence of the bunghole, atmospheric oxygen slowly diffuses into the wine, favoring the stabilization of the coloring matter and the evolution of phenolic composition (Cano-López, López-Roca, Pardo-Minguez, & Gómez Plaza, 2010).

Acetaldehyde, coming from the oxidation of ethanol catalyzed by transition metals or by the coupled oxidation of phenols (Oliveira, Ferreira, De Freitas, & Silva, 2011), plays a major role in these reactions. It acts, in fact, as a bridge for the generation of ethyliden-bridged flavanols polymers (Drinkine, Lopes, Kennedy, Teissedre, & Saucier, 2007) or ethyl bridge-linked pigments and B-type Vitisins (Cano-López et al., 2010). In this context, ellagitannins and ellagic acid released by oak or chestnut woods have been found to be able to reduce the oxidative browning of wines, quickly absorbing dissolved oxygen and modulating the generation of acetaldehyde (Vivas & Glories, 1996). During wood ageing, hence, the presence in wine of constitutive phenols changes depending on a number of factors, including wine type, initial phenolic composition, wood specie and permeability to oxygen.

A number of scientific works have been published on such an argument, the vast majority being focused on French or American oak (*Quercus* spp.), which represent the traditional woods used in cooperage for the ageing of wines and distillates. However, other species such as acacia (*Robinia pseudoacacia*), cherry (*Prunus avium*) or ash (*Fraxinus excelsior*) are increasingly considered for this use (De Rosso, Cancian, Panighel, Dalla Vedova, & Flamini, 2009; Fernández de Simón, Martínez, et al., 2014; Sanz, Fernández de Simón, Cadahia, et al., 2012), due to utilization in local productions (e.g. traditional balsamic vinegar or ciders), their lower costs, or distinctive sensory contribution (Chinnici et al., 2009; Fernández de Simón, Esteruelas, Muñoz, Cadahía, & Sanz, 2009).

For what concerns cherry wood, its use in vinegar or wine production (Cerezo et al., 2008; De Rosso, Panighel, Dalla Vedova,

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Stella, & Flamini, 2009), and the effects of charring on the phenolic composition (Sanz et al., 2010) were recently investigated. In particular, while Cerezo and co-workers (Cerezo et al., 2008) claimed that cherry wood positively contributed to the red fruits notes and the aromatic complexity of vinegars, other researchers reported this wood as the most oxidative between five different wood species (oak, chestnut, acacia, mulberry and cherry), proposing its use only for short ageing periods (De Rosso, Panighel, et al., 2009; Fernández de Simón, Martínez, et al., 2014). It's worth mentioning, however, that in our recent study we found that when compared to oak, cherry wood could promote a faster pigment stabilization, at the same time maintaining the highest color density and the best chromatic attributes of wines (Chinnici, Natali, Sonni, Bellachioma, & Riponi, 2011).

Despite the above mentioned results testify the interest in cherry as a wood suitable for cooperage, scarce detailed information are available on the migration and evolution of phenolic compounds in wines aged in this wood, the only work devoted to this subject being the paper from Fernández de Simón et al. (Fernández de Simón, Sanz, et al., 2014). Due to this, the present work can be considered the finishing of our preliminary findings (Chinnici et al., 2011) and is aimed at i) identifying and quantifying the phenolics constitutive of cherry wood and ii) monitoring the evolution of non-colored phenolic compounds of a sangiovese-merlot blend during the ageing in cherry casks. Containers of two different dimensions (225 L and 1000 L) were used, and the effect of their utilization on the concentration of more than thirty flavonoids and phenolic acids was compared with that provided by oak barriques or the storage in stainless steel.

2. Material and methods

2.1. Reagents

All reagents and solvents were of analytical grade and were purchased from Sigma Adrich (St. Louis, MO USA) or Extrasynthèse (Genay, France). HPLC grade water was obtained with a Simplicity system (Millipore, Bedford, MA, USA).

2.2. Wines and ageing

The samples investigated in this work has been already described in a previous paper (Chinnici et al., 2011). The red wine was a blend of *Vitis vinifera* cv Sangiovese (85%) and Merlot (15%) grapes, harvested in the vintage 2008 by Marchesi dè Frescobaldi winery located in Sieci (Tuscany, Italy), following the traditional vinification protocol. Grapes were destemmed, crushed and sulfur dioxide was added at a dose of 70 mg/kg of grapes. Fermentation took place at 24–26 °C and the cap was immersed twice a day by pumping over. Maceration lasted 20 days after which the must was pressed, and the finished wine (reducing sugars <2 g/L) was obtained. The decanted wine underwent spontaneous malolactic fermentation and wood ageing started in March 2009. For our purposes, two 225 L medium toasted barriques of oak (*Quercus petrae*) or cherry (*P. avium*) wood, together with a further 1000 L cherry wood cask, were used (each ageing condition was carried out in triplicate). All the barrels and casks were obtained from staves seasoned for 24 months. The wines were aged at 80% relative humidity and at temperature conditions ranging between 14 and 16 °C. After 2 months of ageing, sulfur dioxide was adjusted in all the wines to maintain free SO_2 levels ≅ 20 mg/L. Because the trials were arranged also aiming to investigate the anthocyanin concentration, the duration of wood ageing was defined based upon the pigments evolution in wines. Due to this, ageing lasted about 4 months, when cherry barriques caused a decrease in free anthocyanins amount equal to about 80% of the initial value (Chinnici et al., 2011).

2.3. Extraction of non-colored phenolics from cherry wood

Chips of about 1 × 0.5 cm in size and 0.2 cm thick were obtained from five unused staves of medium toasted cherry wood and mixed. Three portions of about 10 g of mixed chips, with a mean total surface of about 20 cm^2, were extracted with 200 mL of synthetic wine (hydroalcoholic solution containing 12% ethanol and 4 g/L tartaric acid) brought to pH 3.4 with 1 M NaOH. Extraction was carried out in the dark at room temperature and lasted 4 months, in this way simulating the extraction conditions of real samples. Extracts were promptly analyzed at the end of the period, without further manipulation except for filtration at 0.45 μm with cellulose filters.

2.4. Analysis of non-colored phenolics in wines and cherry wood extracts

For both wines and cherry wood hydroalcoholic extracts, HPLC separation and identification of non-colored phenolics was performed according to a previously published method (Monagas, Suárez, Gómez-Cordovés, & Bartolomé, 2005), with some modifications. The apparatus was a quadrupole HP 1100 MDS series (Agilent Technologies, Palo Alto, CA), equipped with an autosampler and a diode array UV–Vis detector. The column was a C18 Synergy 4μ hydro RP 80A, 250 × 3.00 mm, operating at 35 °C with a flow of 0.5 mL/min. Fifty microlitres (wood extracts) or 20 μL (wines) of sample were injected. Elution solvents were 2% acetic acid in HPLC grade water (Eluent A) and 2% acetic acid in HPLC grade acetonitrile (Eluent B). Linear gradient for solvent A was as follow: from 98% to 95% in 12 min; 25 min 90%, 32 min 82%, 40 min 80%, 45 min 70%, 50 min 50%, 54 min 20%, 55 min 0% kept for 5 min. Post run was 6 min. The analyses were carried out using an electrospray (ESI) interface operating in negative mode, scanning from 100 *m*/*z* to 1200 *m*/*z* and using the following conditions: drying gas flow, 9.0 L/min; nebulizer pressure, 50 psi; gas drying temperature, 350 °C; capillary voltage, 4000 V; fragmentor voltage, 80 V.

Identification of phenolics was accomplished by comparison of UV and MS spectrum and retention times with those obtained from standard compounds. For compounds lacking of standards, tentative identification was performed by comparing both MS and UV spectrum with literature data. For these compounds, elution order in similar chromatographic conditions (RP-HPLC) reported by other authors was also taken into account and cited all along the text.

Quantification was performed at the UV wavelengths showed in Tables 1 and 2, by using an external calibration curve built with injections of known dilutions. Tentatively identified compounds and phenolics lacking of pure standard were quantified using curve of structurally similar compounds. Therefore, for wine phenolics, cinnamic derivatives were quantified with the corresponding free acid and flavonol glycosides as their respective aglycones. Before injection, wood extracts and wines were filtered at 0.45 μm with a cellulose filter. All analysis were done in duplicate.

2.5. Statistical analysis

Data were subjected to analysis of variance (ANOVA) with post-hoc LSD test and Principal Component Analysis (PCA) by using Statistica 6 (StatSoft Italia srl, Italy) software package.

3. Results and discussion

3.1. Characterization of phenolics extracted from cherry wood

In order to characterize and deepen the contribution of cherry wood on the phenolic composition of wines, chips obtained from new cherry staves were extracted using a model wine solution.

Table 1
Phenolic compounds identified in cherry wood extracted in model wine for 4 months.

	Compound	rT	λ quantification (nm)	λ max (nm)	$(M-H)^-$	Amount (μg/g wood)
Flavanols						
2	Procyanidin B1	22.14	280	278	577	7.90
3	B-type procyanidin trimer[a]	22.55	280	278	865	18.4
4	(+)-Catechin	25.16	280	278	289	766
5	Procyanidin B2	30.25	280	278	577	1.95
6	(−)-Epicatechin	34.08	280	278	289	16.6
8	B-type procyanidin dimer[a]	36.95	280	277	577	124
	Sum procyanidins					*935*
Flavanonols						
7	Taxifolin	36.30	280	288	303	2.88
9	Aromadendrin	41.16	280	290	287	32.4
	Sum flavanonols					*35.3*
Flavanones						
10	Flavanone derivative[b]	42.49	280	288, 332	287	2.07
11	Flavanone derivative[b]	45.01	280	290	287	3.59
12	Eriodictyol	48.06	280	288, 332	287	36.2
13	Naringenin	51.55	280	288, 330	271	6.64
15	Hesperetin	52.45	280	288, 330	301	4.20
16	Flavanone derivative[b]	53.97	280	290, 338	271	27.6
19	Sakuranetin	57.40	280	290, 332	285	99.4
20	Pinocembrin	57.60	280	290, 332	255	191
	Sum flavanones					*371*
Flavonols						
14	Quercetin	52.19	370	254, 371	301	3.04
17	Kaempferol	54.87	370	367	385	0.96
	Sum flavanols					4.00
Others						
1	Protocatechuic Acid	11.84	280	260, 292	153	4.25
18	Unknown	54.98	280	256, 285	–	–
21	Chrysin	57.76	280	267, 325	253	71.3

[a] Quantified as (+)-catechin.
[b] Quantified as eriodictyol.

Apart from oxygen permeation, other extraction conditions such as temperature, duration and wood to wine ratio were as close as possible to the ones the wines actually underwent.

Fig. 1 shows a representative chromatogram of cherry wood phenolics, obtained after 4 months of extraction. Fifteen compounds were identified by comparing their spectral features with those of standard compounds and literature data while, for other 5 compounds, a tentative identification, based on UV and MS spectra, has been proposed (Table 1). A further compound (#18) with a relevant peak at 54.98 min also emerged but, due to the unexpected suppression of MS fragmentation, its identification was not possible.

Flavanols and procyanidins were the main chemical classes found in cherry wood, with a total concentration of about 0.9 mg/g of wood (Table 1). Six flavanols were detected, including compounds 2, 4–6 (which have been identified by means of standards), one dimer (# 8) and a trimer (#3) the both elucidated thanks to MS and UV spectrum. Among them, (+)-catechin and one of its dimer represented around 90% of the entire flavanols concentration. In a medium toasted cherry, Sanz et al. (Sanz et al., 2010) reported (+)-catechin as the unique flavanol present, at amounts around 150 μg/g. However, when compared to oak or chestnut, cherry wood has been described as the most dramatically influenced by charring (Soares, Garcia, Costa Freitas, & Cabrita, 2012), and in a further paper by Sanz and colleagues (Sanz, Fernandez de Simon, Cadahia, et al., 2012), up to 10 mg/g of procyanidins were found in a lightly toasted cherry sample. The exact definition of the wood toasting level only depends on cooperages, and significant differences between casks at the same nominal level of charring could be not unusual.

Flavanones represented the second chemical class, in terms of quantity, extracted from the cherry wood. Five compounds were identified: eriodictyol, naringenin, hesperetin, sakuranetin and pinocembrin. Reports on the presence of these biological markers in the wood of several *Prunus* species dates back some decades, and have been mainly aimed to taxonomic (Hasegawa, 1957; Nagarajan & Parmar, 1977) (Nagarajan & Parmar, 1977) or both antioxidant and antimicrobial-related purposes (McNulty et al., 2009). More recently, two already cited studies devoted to woods employable in cooperage (Sanz et al., 2010; Sanz, Fernández de Simón, Esteruelas, et al., 2012) identified eriodictyol, naringenin, hesperetin and pinocembrin in seasoned and light toasted cherry wood, but not sakuranetin. The occurrence of this latter compound, whose UV and (M−H) features are the same as its isomer isosakuranetin, was unambiguously ascertained by fortification experiments and confirms the findings of other researchers who indagued on *P. avium* heartwood (McNulty et al., 2009; Vinciguerra, Luna, Bistoni, & Zollo, 2003). Three further compounds (compounds #10, #11 and #16) displayed UV spectrum and MS fragmentation referable to flavanone aglycones (Fabre, Rustan, de Hoffmann, & Quetin-Leclercq, 2001) and were tentatively assigned accordingly. It's worth noting that, in cherry heartwood, Sanz et al. (2010) described two molecules with very similar characteristics and elution order, which they identified as polyhydroxyflavanones.

Other flavonoids identified in the extracts at very low amounts (<3 μg/g) comprised quercetin and kaempferol, which are synthesized by the plant metabolism starting from taxifolin (compound #7) and aromadendrin (compound #9) (Martens et al., 2003). All these compounds have been already reported in the heartwood of various *Prunus* species (Hasegawa, 1957; Hasegawa & Shirato, 1957).

Chrysin was the only flavone found in our extracts at quite an high concentration (around 70 μg/g). This compound has been described as one of the main flavonoid present in recently felled *P. avium* heartwood (Nagarajan & Parmar, 1977; Vinciguerra et al., 2003), at amounts of about 200 μg/g (Hasegawa, 1957). On the other hand, other researchers did not report chrysin among the flavonoids extracted from seasoned or toasted cherry wood (Sanz et al., 2010), suggesting the natural variability of the wood as raw material for cooperage.

Overall, medium toasted cherry wood was found to be mainly composed of flavanols, condensed tannins and flavanones, hence substantially different from other woods widely used in cooperage (oak and chestnut above all) where gallic acid, hydrolyzable tannins and ellagic acid prevail (Alañón & Castro-Vázquez, 2011; Doussot, De Jéso, Quideau, & Pardon, 2002).

3.2. Evolution of non-colored phenolics during wood ageing

In our study, the influence of the ageing in cherry barriques (225 L) and barrels (1000 L) on the evolution of non-colored phenolics, has been compared to that obtained from the ageing in oak barriques or the storage in stainless steel containers.

Table 2 shows some analytical features of the low MW phenolics quantified in the wines, together with their evolution during the storage or the ageing in the two different botanical species.

Identification of compounds in wines was based on UV and MS spectra as well as RP-HPLC elution order as also reported in other published papers (Monagas et al., 2005; Sanz, Fernández de Simón, Esteruelas, et al., 2012; Sun, Liang, Bin, Li, & Duan, 2007).

Benzoic acids tended to decrease with time, particularly from the second month onward, mainly due to the diminution of ethyl gallate, whose amount in final wines dropped to 30–60% of the initial concentration (Table 2). This could be due to the hydrolysis of this ester to give gallic acid which, infact, showed an overall

Table 2
Chromatographic features of phenolics identified in red wines and their amounts (mg/L) during ageing.

Compound	λ quantification nm	$(M-H)^-$	Initial wine	2 months				4 months			
				Steel	Oak 225 L	Cherry 225 L	Cherry 1000 L	Steel	Oak 225 L	Cherry 225 L	Cherry 1000 L
Benzoic acids											
Gallic acid	280	169	70.2 a	65.9 ab	66.7 ab	64.1 b	57.4 c	70.8 a	68.0 a	64.7 b	66.0 ab
Ethyl gallate	280	197	48.7a	50.0 a	47.0 ab	33.1 b	33.6 b	29.2 bc	25.9 c	14.1 d	15.0 d
Protocatechuic acid	256	153	4.61 a	4.45 a	4.20 ab	4.27 a	2.83 c	3.85 b	3.74 b	2.72 c	3.61 b
Vanillic acid	280	167	1.68 a	1.49 ab	1.63 a	1.37 ab	1.15 b	1.02 b	1.81 a	1.14 b	0.89 c
Ellagic acid	350	301	4.35 b	4.14 b	4.53 b	1.39 e	3.06 d	3.40 c	7.66 a	3.44 c	4.39 b
Sum			**129.6 a**	**126.0 a**	**124.0 a**	**104.2 b**	**98.0 c**	**108.3 b**	**107.1 b**	**86.1 d**	**89.9 d**
Cinnamic acids											
Caffeic acid	324	179	7.18 a	6.75 ab	6.72 ab	6.46 b	5.79 d	6.99 a	6.16 bc	5.82 d	6.05 cd
p-cumaric acid	310	163	1.47 a	1.40 a	1.46 a	1.34 ab	1.52 a	0.97 bc	0.89 c	1.08 bc	1.05 bc
Caftaric acid	324	311	40.3 a	37.7 ab	38.8 a	36.9 ab	32.8 b	38.9 a	36.6 ab	36.3 ab	37.3 ab
GRP	324	616	7.66 a	7.08 b	7.08 b	6.92 b	6.03 c	6.94 b	6.17 c	5.96 d	6.47 bc
c-Cutaric acid	310	295	2.84 a	2.64 ab	2.51 b	2.57 b	2.26 c	2.54 b	2.39 bc	2.44 bc	2.53 b
t-cutaric acid	310	295	8.52 a	8.00 b	7.90 b	7.82 b	7.03 c	7.90 b	7.97 b	7.63 bc	7.97 b
Fertaric acid	324	325	12.5 a	11.8 ab	13.1 a	12.6 a	10.3 b	13.1 a	13.2 a	12.2 ab	13.0 a
Ethyl cumarate	324	191	0.10 a	0.11 a	0.10 a	0.12 a	0.10 a	tr[a]	tr	tr	tr
Sum			**80.6 a**	**75.5 ab**	**77.7 a**	**74.8 ab**	**65.8 c**	**77.3 a**	**73.4 b**	**71.4 b**	**74.3 ab**
Flavanols											
(+)-Catechin	280	289	59.7 a	57.8 a	56.1 a	53.0 ab	50.5 b	51.6 b	42.7 c	21.4 e	32.6 d
(−)-Epicatechina	280	289	55.4 a	53.7 ab	52.0 b	45.2 c	43.5 cd	45.3 c	38.6 d	20.5 f	27.5 e
Procyanidin B1	280	577	87.7 a	89.1 a	82.8 b	75.6 c	76.1 c	82.5 b	66.5 d	26.7 f	44.7 e
Procyanidin B2	280	577	93.3 a	90.5 ab	86.9 b	73.9 d	72.8 d	82.2 c	68.4 e	27.7 g	43.1 f
Sum			**296.0 a**	**291.1 a**	**277.8 b**	**247.7 d**	**242.9 d**	**261.6 c**	**216.2 e**	**96.3 g**	**147.8 f**
Flavonols											
Isorhamnetin-3-Glucoside	350	477	2.12 a	2.09 a	2.07 a	1.83 ab	1.67 b	2.21 a	2.06 a	1.74 b	1.85 ab
Isorhamnetin	350	315	1.25 b	1.24 b	1.20 b	1.16 bc	1.14 bc	1.45 a	1.40 a	1.21 b	1.28 b
Kaempferol	350	285	1.59 ab	1.61 ab	1.33 b	1.43 b	1.38 b	1.72 a	1.60 a	1.28 b	1.52 ab
Myricetin-3-Glucoside	350	479	5.39 a	4.86 ab	4.91 ab	2.88 d	2.67 d	5.15 a	4.93 ab	3.43 c	4.41 b
Myricetin	350	317	5.69 a	5.94 a	5.37 ab	5.77 ab	5.13 b	4.87 c	4.47 d	3.14 e	4.17 d
Quercetin	350	301	18.0 a	18.6 a	14.6 c	16.7 b	14.3 c	17.6 a	14.6 c	8.55 e	12.1 d
Quercetin-3-Glucoside	350	463	1.27 ab	1.40 a	1.39 a	1.23 ab	0.52 d	1.13 b	0.79 c	0.64 cd	0.77 c
Quercetin-3-Glucuronide	350	477	7.28 a	7.34 a	7.28 a	6.57 b	5.57 c	7.22 a	6.75 b	4.44 d	5.89 c
Sum			**42.5 a**	**43.0 a**	**38.1 b**	**37.6 b**	**32.4 c**	**41.3 a**	**36.6 b**	**24.4 d**	**31.9 c**
Other phenolics											
Tyrosol	280	137	53.8 a	50.7 ab	57.1 a	49.4 b	50.9 ab	49.3 b	55.5 a	52.6 ab	51.4 ab
t-Resveratrol	310	227	1.09 a	1.02 a	1.01 a	0.94 a	1.08 a	1.07 a	1.04 a	0.69 b	0.98 a
t-Resveratrol glucoside	324	389	6.20 a	5.17 c	4.10 d	5.01 c	4.43 d	5.88 b	5.49 bc	5.33 bc	5.61 b
Eriodictyol	280	287	n.d.[b]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.09 a	0.08 a
Flavanone derivative	280	271	n.d.	n.d.	n.d.	0.31 b	0.20 c	n.d.	n.d.	0.51 a	0.49 a
Sakuranetin	280	285	n.d.	n.d.	n.d.	0.86 b	0.59 c	n.d.	n.d.	2.21 a	1.97 a
Pinocembrin	280	255	n.d.	n.d.	n.d.	1.44 b	0.92 c	n.d.	n.d.	1.72 a	2.59 a
Chrysin	280	253	n.d.	n.d.	n.d.	0.11 c	0.13 c	n.d.	n.d.	0.71 a	1.07 a

In each line, different letters denote significant differences at $p \geq 0.05$.
[a] The text "tr" stands for "traces".
[b] n.d. = not detected.

stability during ageing, likely following the variable equilibrium between oxidative phenomena and re-generation from hydrolysis. The usage of cherry wood drove to a significantly faster disappearance of ethyl gallate, independently from the container used (barrique or larger barrel).

As expected, after 4 months, the wines stored in oak had the highest amount of ellagic acid, due to the cession from that wood (Chira & Teissedre, 2013; Vivas, 2000). Interestingly, cherry wood appeared to promote an initial decrease of ellagic acid in the wines (see values at two months, in Table 2) followed by an augmentation, in both the kind of containers. Taking into account the absence of ellagic acid in our cherry staves extracts (Table 1), it could be supposed that a certain amount of ellagitannins could have been leaked from the untoasted wood used for barrel heads. In fact, little amounts of both ellagitannins and ellagic acid were found in seasoned cherry wood (Alañón & Castro-Vázquez, 2011), corroborating our hypothesis. However, it should be mentioned that, for this and other phenolic compounds, other authors reported the great influence exerted by the grape cultivar used in winemaking (Fernández de Simón, Sanz, et al., 2014).

Cinnamic acids underwent to a general little decrease during ageing, with no clear differences between the alternative woods or containers (Table 2). For cinnamoyl tartaric acids, the same results were found by De Rosso et al. (De Rosso, Panighel, et al., 2009) who compared 5 different wood species usable in cooperage, including cherry. On the contrary, Fernandez de Simon et al. (Fernández de Simón, Sanz, et al., 2014) described a higher decrease of caftaric and coutaric acids in wines aged in cherry barrels if compared with oak barrels. The change of cinnamates amounts all along the ageing period is not so obvious and depends on a number of factors including wood specie, grape origin and cultivar (Sanz, Fernández de Simón, Esteruelas, et al., 2012) or the temperature of storage and the entity of hydrolysis of the cinnamic derivatives (Gutiérrez, Lorenzo, & Espinosa, 2005). Furthermore, their participation in oxidative reactions is widely known (Oliveira et al., 2011). Contrasting data could hence be found in literature, where increasing amounts of cinnamic acids (Ginjom, D'Arcy, Caffin, & Gidley, 2011) are opposed to their complete disappearance during ageing (Gutiérrez et al., 2005). Our dataset suggests, however, that independently from the storage conditions, *p*-cumaric acid and ethyl cumarate clearly tended to decrease during ageing.

The class of flavanols was the one with the largest quantitative variations recorded during the ageing (Table 2). In the samples

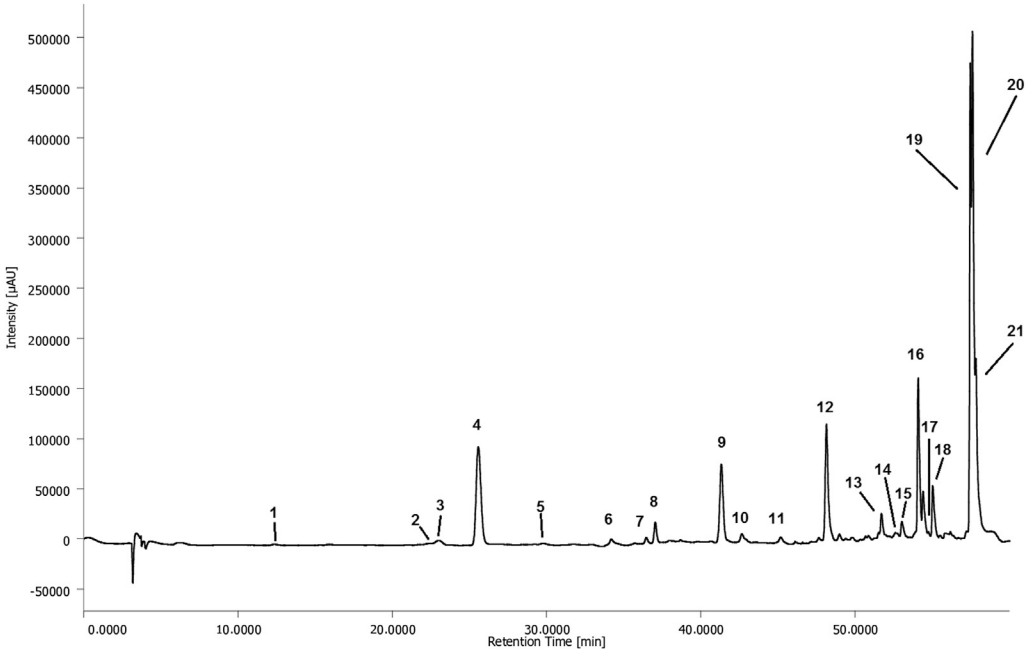

Fig. 1. HPLC chromatogram, detected at λ 280 nm, of cherry wood extracted for 4 months in model wine solution. For peak identification refer to Table 1.

stored in steel, likely because of the limited exposure to oxygen, a significant decrease of all these compounds was only recorded after 4 months of conservation. However, wood ageing caused a faster diminution of these low MW phenols, with cherry barriques clearly promoting the highest rate of disappearance followed by cherry barrels and oak barriques. This done is consistent with the findings of De Rosso et al. (De rosso, Panighel et al., 2009), but contrasts with the data from other researcher (Fernández de Simón, Sanz, et al., 2014), who reported significant higher concentrations of (+)-catechin in red wines aged in cherry barrels compared to those aged in chestnut, acacia, ash or oak barrels. The decline of flavanols and dimeric procyanidins during ageing could be due to both oxidation and polymerization phenomena, where oxygen permeation and phenolics leakage from wood play a major role (Vivas, 2000; Vivas & Glories, 1996). Ellagitannins extracted from oak, for example, were found to regulate the oxido-reductive mechanisms thanks to their rapid oxidation, favoring the flavanol/anthocyanins or flavanol/flavanol condensations while reducing the oxidative loss of catechins and stabilizing the wine color (Vivas & Glories, 1996). Unlike oak, however, cherry wood lacks in ellagitannins and no information are available on the effects of the main constituents of this wood (flavanones, flavanonols and flavones) on the phenolic evolution of red wines. Interestingly, a very high oxidizability of cherry wood extracts has been already reported (De Rosso, Cancian, et al., 2009) and data previously published by our research group suggest that in cherry aged wines, a considerable amount of flavanols could be implied in acetaldehyde mediated condensation, contributing in enhancing pigments stabilization, deepening the crimson color and increasing the level of polymerized tannins (Chinnici et al., 2011).

Regarding flavonols (Table 2), in stainless steel stored samples their total amount was unchanged at the end of the investigated period. Once again, the practice of ageing wines in wood caused a significant decrease of flavonoids, with the order cherry barrique > cherry barrel > oak barrique. Confirming other published studies (Castellari, Piermattei, Arfelli, & Amati, 2001; Sanz, Fernandez de Simon, Cadahia, et al., 2012), quercetin and his glycosides were greatly affected by ageing conditions while other compounds, such as kaempferol and isorhamnetin-3-glucoside, were relatively unaffected. As already found by others (De Rosso, Panighel, et al., 2009), in our samples, because of the stronger oxidative environment, cherry wood promoted up to 34% decrease in flavonols with respect to oak. In addition, as in the case of other phenolic classes seen before, the use of larger casks could mitigate the extent of oxidative phenomena, likely because of their lower wood surface/volume of wine ratio.

In Fig. 2, the HPLC traces of two wines sampled after 4 months of storage in oak (panel A) or cherry wood (panel B) are depicted. In that figure, the differences in Malvidin-3-glucoside content of the two samples could be appreciated as well by comparing the height of the peak flagged with asterisks. As can be noted, the wine aged in cherry is clearly distinguishable from the other due to the presence of five additional peaks referring respectively to eriodictyol, a flavanone derivative, sakuranetin, pinocembrin and chrysin. The content of these compounds, evidently extracted from the wood, increased during the ageing period and did not show significant differences among barriques and larger casks (Table 2). Only one other study focused on the identification of possible non-volatile phenolic markers of ageing in cherry wood (Fernández de Simón, Sanz, et al., 2014). Those authors found that 6 compounds, including taxifolin, prunin, aromadendrin, eriodictyol, naringenin and isosakuranetin, could be regarded as chemical markers of the use of this botanical specie in winemaking. Our data suggest that, probably because of both the natural variability of woods for cooperage and differences in seasoning and toasting practises, other phenolic compounds such as sakuranetin, pinocembrin and chrysin may further distinctly characterize red wines stored in cherry barrels.

An overview of the features of wines aged at different conditions is provided in Fig. 3, where the results of PCA carried out on the entire dataset are shown as a biplot. Principal components 1 and 2 explained 62.3% of the total variance. Overall, as expected, the figure suggests that the longer the ageing time, the larger the

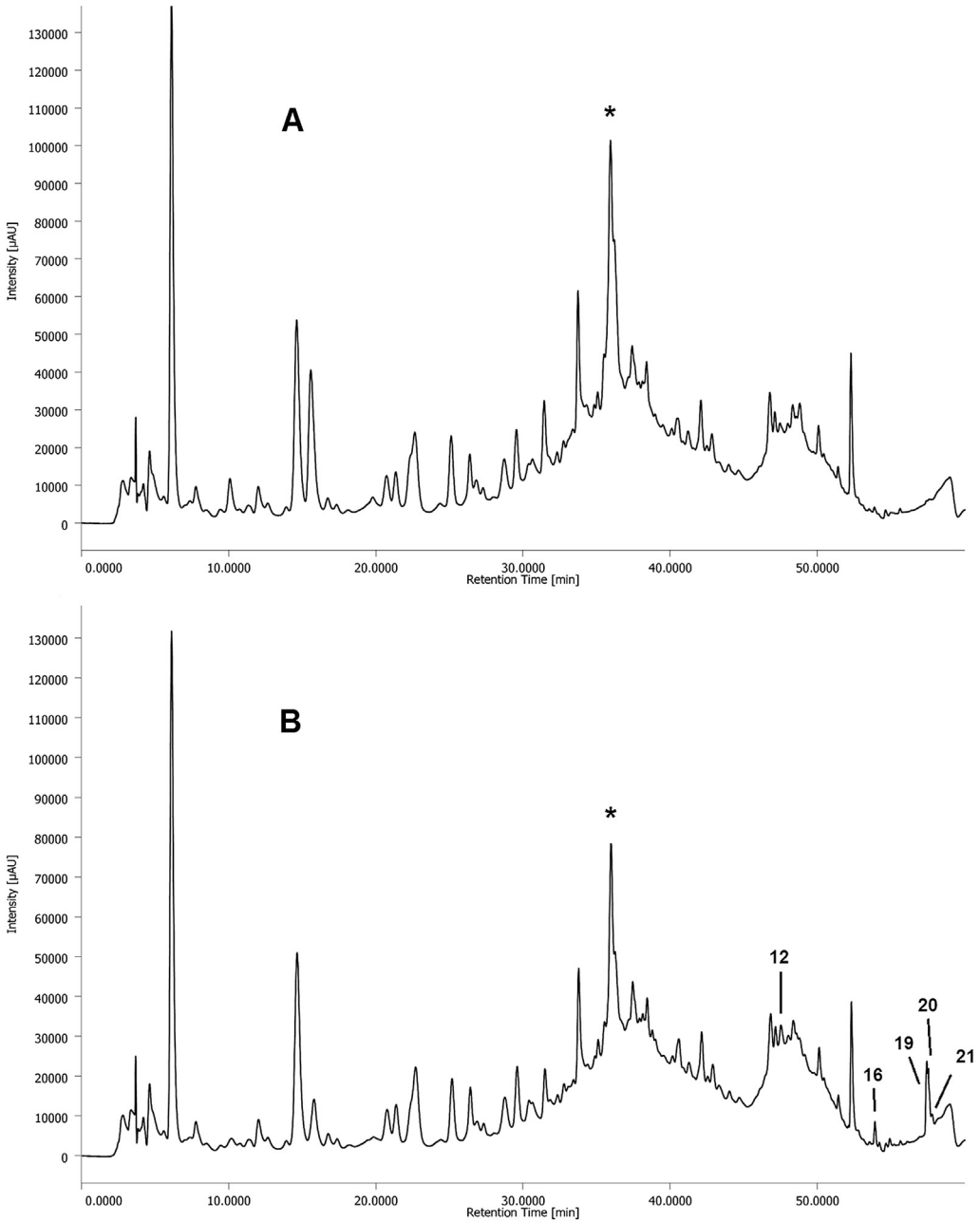

Fig. 2. HPLC chromatogram, detected at λ 280 nm, of wines at the end of the ageing period (4 months). Legend: (A): Oak aged wine; (B): Cherry aged sample; *: Unresolved peaks of peonidin-3-glucoside and malvidin-3-glucoside. Peaks are numbered according to Table 1.

differences among wines. On the first principal component (PC1), the samples stored in cherry wood for 4 months (whether in barriques or casks) are clearly separated from all the other samples. Those wines were the only containing the phenolics extracted from cherry staves. On the same PC1, the remaining wines demonstrated to be poorly discriminated each other, being characterized by higher amounts of flavanols (particularly procyanidins and (+)-catechin). PC2 roughly separated the samples according to the storage time. *p*-coumarate and ethyl coumarate were positively correlated with the samples stored for 2 months, while flavonols (kaempferol and isorhamnetin) and *t*-resveratrol glucoside contributed in separating wines aged for 4 months as a function wood species.

4. Conclusions

Phenolics extracted from cherry wood have been elucidated to be mainly composed of flavanols, flavanones, flavones and flavanonols. During the ageing, some of these compounds (eryodictiol, sakuranetin, pinocembrin and chrysin) are progressively transferred to the wine, and could be regarded as phenolic markers of this winemaking step.

If compared with oak, cherry wood promoted a faster evolution of wine constitutive phenols. This evolution was characterized by a greater reduction in flavanols and flavonols, the formers being involved in condensation phenomena able to stabilize wine color. The use of larger casks (1000 L) mitigated the effects of cherry

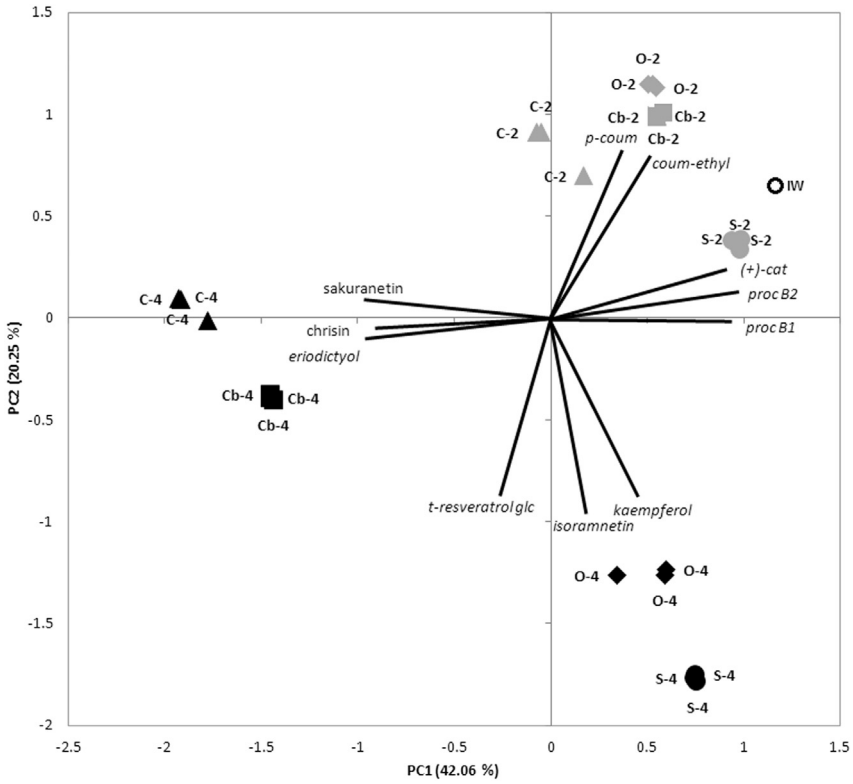

Fig. 3. Biplot of the Principal Component Analysis (PCA) showing the contribution of the variables and distribution of wines in the two-dimensional coordinate system defined by the first two principal components. IW, Initial Wine; S, Stainless steel; O, Oak wood; C, Cherry wood (225 L); Cb, Cherry wood (1000 L); 2, samples after 2 months of ageing; 4, samples after 4 months of ageing; *p*-coum, *p*-coumaric acid; coum-ethyl, ethyl *p*-coumarate; (+)-cat, (+)-catechin; proc B1, procyanidin B1; proc B2, procyanidin B2.

wood, confirming the pivotal role played by the oxygen transfer in the phenomena observed.

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