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Changes in phenolic composition of red wines aged in cherry wood

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(+)-catechin (PubChem CID: 9064)
Sakuranetin (PubChem CID: 73571)
Pinocembrin (PubChem CID: 68071)
Chrysin (PubChem CID: 5281607)
Eriodictyol (PubChem CID: 11095)
Procyanidin B2 (PubChem CID: 122738)

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.