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Comparative Changes in Color Features and Pigment Composition of Red Wines Aged in Oak and Cherry Wood Casks

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ABSTRACT: The color features and the evolution of both the monomeric and the derived pigments of red wines aged in oak and cherry 225 L barriques have been investigated during a four months period. For cherry wood, the utilization of 1000 L casks was tested as well. The use of cherry casks resulted in a faster evolution of pigments with a rapid decline of monomeric anthocyanins and a quick augmentation formation of derived and polymeric compounds. At the end of the aging, wines stored in oak and cherry barriques lost, respectively, about 20% and 80% of the initial pigment amount, while in the 1000 L cherry casks, the same compounds diminished by about 60%. Ethyl-bridged adducts and vitisins were the main class of derivatives formed, representing up to 25% of the total pigment amount in the cherry aged samples. Color density augmented in both the oak and cherry wood aged samples, but the latter had the highest values of this parameter. Because of the highly oxidative behavior of the cherry barriques, the use of larger casks (e.g., 1000 L) is proposed in the case of prolonged aging times.

KEYWORDS: Cherry wood, oak, red wine, wine aging, anthocyanins, color

INTRODUCTION

Together with aroma, wine color is one of the most relevant features perceived by consumers. It furnishes indirect information on the age of the product and its storage history and influences the overall acceptance of the wine.

The pivotal role played by anthocyanins in determining the color of wine is well established. Once extracted from the grape skins to the must, their concentration begins to decrease because of polymerization, oxidation, or precipitation phenomena. Furthermore, they may undergo a series of reactions with other wine constituents. In fact, apart from the absorption by yeast cells,[1] which takes place at the end of maceration, during fermentation, storage, and aging, monomeric anthocyanins may react with pyruvic acid,[2] acetaldehyde[3] (giving rise to A-type and B-type vitisins, respectively), vinylphenols,[4] or procyanidins,[5] forming adducts which are characterized by a new pyran ring generated by the addition of the former compounds to carbon in position 4 and hydroxyl group in position 5 of the anthocyanin. The pyranoanthocyanins formed in such a way share a hypsochromic shift of the λ maximum with respect to native anthocyanins, yielding an orange nuance to wines.[6] Other reactions producing a qualitative change in pigments are those which involve their condensation with flavanols, either directly[7] or mediated by acetaldehyde.[8] Once again, these newly formed compounds have demonstrated to have a potential influence on the color of wines, providing a bluish-red hue, due to the bathochromic shift of their visible absorption maxima.[6,8,9]

In addition, it is worth noting that a common chromatic characteristic of both pyranoanthocyanins and condensed derived pigments is the greater resistance to bisulphite bleaching and pH changes[10] that could further enhance their overall sensorial importance.

Wood aging is a common practice in the production of high quality red wines. Contact with wood can promote migration into the wine of a number of compounds which may positively influence the complexity and intensity of flavor and aroma.[11,12] Furthermore, during aging, because of both the porosity of the wood fibers and the presence of the bunghole, molecular oxygen slowly diffuses into the wine, favoring the stabilization of the coloring matter and the evolution of the phenolic composition.[11,13]

This latter phenomena are believed to be mainly due to the formation of relevant amounts of acetaldehyde (coming from the oxidation of ethanol catalyzed by transition metals or through the coupled oxidation of phenols)[14,15] which in turn acts as a bridge for the generation of ethylidene-bridged flavan-3-ols,[16] the already mentioned ethyl bridge-linked compounds, or the B-type Vitisins.

The large majority of the published work aiming to investigate the influence of wood aging on the pigment composition of red wines has been focused on French or American oak (*Quercus* spp.), which represents the traditional species used in cooperage for the aging of wines and distillates.

However, other species such as chestnut (*Castanea sativa*), acacia (*Robinia pseudoacacia*), cherry (*Prunus avium*), or ash (*Fraxinus excelsior*) are increasingly considered for such use[17,18] due to their lower costs, unique sensory contribution, or local use in specific productions (e.g., traditional balsamic vinegar or ciders).[19,20]

In particular, the sensory findings of Cerezo et al.[21] on aged wine vinegars confirmed the preliminary and not yet published results of our group on the intriguing contribution of cherry wood to the aromatic complexity of red wines, which were characterized by significant increases in cherry and red fruits notes, together with an improved overall general impression. Undoubtedly, depending on the toasting level, the volatile

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composition of the hydroalcoholic extract of this wood has been reported to be very different from the oak wood extract. It is in fact characterized by higher amounts of benzaldehyde, methyl syringate, benzoic acid, and 3,4,5-tryhydroxyphenol and lower concentrations of volatile aldehydes,[18] these latter typically found in charred oak wood. At the same time, De Rosso et al.,[17] by comparing five different wood species (oak, chestnut, acacia, mulberry, and cherry), reported that cherry wood promoted the most oxidative environment, and proposed its use only for a short aging period, which is in partial contrast to other reports on acetification kinetics[22] where acacia wood was even more oxidative than cherry or oak wood.

In view of its usage in cooperage, fuelled by the impressive aromatic footprint and the scarcity of published studies, we decided to undertake the present work in order to elucidate the impact of cherry wood on the oxidative evolution of both the color and pigments of red wines. To this aim, the native anthocyanins and the related pigment derivatives of a red wine aged for 4 months in both 225 L and 1000 L cherry wood barrels were monitored and compared with an oak-aged wine. The color features of the resulting products were also evaluated and discussed in light of their pigment composition.

MATERIALS AND METHODS

Reagents. All reagents were of analytical grade. HPLC grade water was obtained by means of a Simplicity system (Millipore, Bedford, MA, USA). Malvidin-3-*O*-glucoside chloride (>95% purity) was from Extrasynthese (Genay, France).

Wines and Aging. The wine used in the experiment was a blended red wine made from *Vitis vinifera* cv Sangiovese (85%) and Merlot (15%) grapes, vinified in the vintage 2008 by the Marchesi dè Frescobaldi winery located in Sieci (Tuscany, Italy), following the traditional vinification protocol. After grape destemming and crushing, 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 aging started in March 2009. For our purposes, two 225 L medium toasted barriques of oak (*Quercus Petrae*) and cherry (*Prunus avium*) wood, together with a further 1000 L cherry wood cask (light toasting), were used (each aging 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 14−16 °C. One thousand liter stainless steel containers, held under the same conditions as the wooden casks, were also arranged and used as reference storage conditions. Because the present work aimed to investigate anthocyanin evolution, the duration of wood aging was defined upon the basis of the pigment decline in wines. As discussed in the following sections, after 125 days of aging, the total amount of anthocyanins in the wines stored in cherry barriques decreased to about 20% of the initial value, and acylated anthocyanins were almost totally absent. Because of this, the experiment lasted about 4 months when, however, the sensory contribution of cherry wood (in terms of red fruit notes) was largely perceptible.

General Enological Parameters and Dissolved Oxygen. Titratable acidity (TA), volatile acidity (VA), pH, reducing sugars, alcoholic strength, and total and free SO_2 were determined according to the methods proposed by OIV.[23] Total phenolics (TP) determination was carried out by means of the Folin−Ciocalteau reagent,[24] while tannins (T) were estimated following the methods of Ribereau−Gayon and Stonestreet.[25]

Acetaldehyde was analyzed according to the method outlined by the AOAC.[26] A Fisons gas-chromatograph 8000 series (Milano, Italy) with a flame ionization detector and a packed column of 23% Carbowax 1500 (w/w) on Chromosorb W (60−80 mesh) were used.

During aging, the amount of oxygen dissolved in the wines was monitored weekly by using an HQ30D Flexy meter (Hach Lange, Milan, Italy) and immersing the probe directly in the center of the container or the cask through the bunghole. Within each replicated measurement (n = 3), the coefficient of variation (CV%) spanned between 2.5% and 9.2%. Each measurement lasted not more than 60 s.

Pigment Analysis. Pigment analysis was carried out by RP-HPLC/DAD, with a method previously published by our group.[9] The apparatus was a Jasco (Tokyo, Japan) equipped with a Rheodyne valve (Cotati, CA), a 50 μL loop, a photodiode detector, and an oven. The column was a Phenomenex (Torrance, USA) Synergy Hydro-RP 80A 25 cm × 3.0 mm ID. The elution solvents were 10% formic acid in HPLC grade water (solvent A) and 10% formic acid, 45% CH_3CN, and 45% HPLC grade water (solvent B). Gradient elution was as follows: from 85% to 70% A in 17 min, then to 27% A at 45 min, then 0% A at 48 min, and then 85% A at 50 min. The column was thermostatted at 30 °C with a flow of 0.47 mL/min. Quantification was performed at 520 nm (except for B-type vitisins which were quantified at 490 nm), and the concentration of each compound was expressed as malvidin-3-glucoside equivalents (mg/L). Wines were filtered at 0.45 μm and directly injected. Identification of compounds was performed by means of the elution order and the comparison of UV−vis spectra with those already elucidated by RP-HPLC/ESI-MS.[9]

Color Measurements. Absorbance measurements were made with a Jasco 810 spectrophotometer (Tokyo, Japan), and samples were adjusted to pH 3.6 and filtered (0.45 μm) with cellulose filters before analysis. Apart from color intensity (I) and shade (N),[23] other color indexes were calculated after some modifications of the methods proposed by Somer and Evans[27] and Boulton et al.[28] as briefly described below.

WCP: This represents the color of pigments after their conversion into the flavylium form. Wine (0.5 mL) has been diluted in 20 mL of 1 M HCl. After 30 min, the absorbance (at 520 nm) was measured in a 10 mm cuvette and corrected for dilution.

WC: This is the color due to anthocyanins after their release from bisulphite adducts. Fifty microliters of 10% acetaldehyde was added to 5 mL of wine, and after 45 min, absorbance (at 520 nm) was measured in a 1 mm cuvette and corrected to a 10 mm pathway.

CAW (chemical age of wine): this index represents the ratio (%) of the color due to polymeric pigments. The calculation proposed by Somers and Evans[27] was adopted.

CDD (color due to derivatives): This parameter expresses the color amount due to derived pigments and colored polymers and was calculated using both the spectrophotometric and the HPLC data, with the following formula:

$$\mathrm{CDD}\ (\%) = (\mathrm{WCP} - \mathrm{FAA})/\mathrm{WCP} \times 100$$

where FAA is the absorbance of free anthocyanins as obtained from the HPLC analysis (as mg/L) converted by applying a molar absorptivity value (ε) of 20200 L M^{-1} cm^{-1} [29] and a MW of 529.

Statistical Data Treatment. Statistical treatment of data was carried out by using the package STATISTICA 6, StatSoft Italia srl.

RESULTS AND DISCUSSION

Evolution of Dissolved Oxygen into the Samples. Figure 1 depicts the evolution of dissolved oxygen (DO) in the four different containers, during the aging period. In Figure 1, time 0 represents the oxygen level of the initial wine. Because of the filling operations and the permeation through the staves, DO rapidly increased in all of the wood aged samples reaching, in the

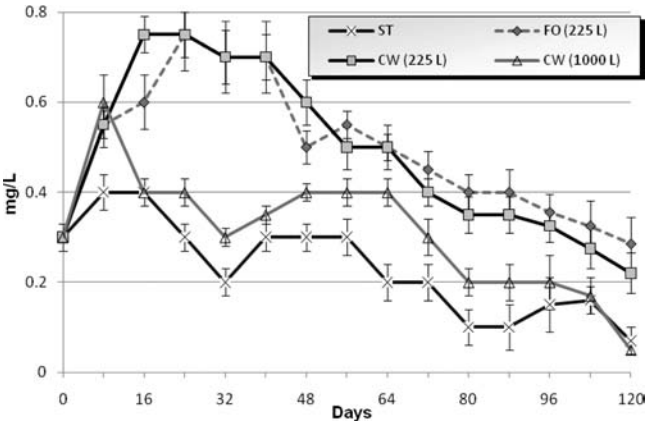

Figure 1. Evolution of dissolved oxygen in the wines during aging (SD is given for each replicated measurement). ST, stainles steel tank; FO, French oak; CW, cherry wood.

225 L casks, values as high as 0.75 mg/L after 20 days, whatever the type of wood. A constant decreasing trend followed and, at 120th day, oxygen levels were about 0.300 mg/L for both the oak and cherry 225 L barrels and 0.050 mg/L for the 1000 L cherry wood cask. Thus, as expected, with time the oxygen-consuming phenomena prevailed on the oxygen dissolution kinetics, generating the descending profile of DO described in Figure 1. It has been argued that, in red wines, once the oxygen dissolution kinetic becomes lower than the consumption kinetic, the levels of dissolved oxygen may stabilize around 0.010−0.040 mg/L.[30] In the parity of other conditions (e.g., type of wine, storage temperature, and humidity, etc.), this equilibrium is mainly ruled by the wood surface to wine volume ratio, with the smaller 225 L casks having both a higher ratio and dissolution rate than the 1000 L barrels. This fact may justify the flatter descending profile of 225 L casks (Figure 1).

By looking at the same figure, it also appears that, during the entire storage time, the level of DO in 225 L French oak barrels was very similar to that of the 225 L cherry wood casks.

General Oenological Parameters. The evolution of general oenological parameters are shown in Table 1. Parameters such as pH, TA, VA, and reducing sugars did not vary significantly all along the aging period, and after 4 months, the final wines had unchanged values with respect to the initial wine.

In wood aged wines, total SO_2 decreased since the first month of storage, and the samples in 225 L cherry wood casks showed a somewhat higher reduction trend if compared to all the other samples. It is worth mentioning that, after 60 days of aging, a correction of total SO_2 (aimed to attain values of free SO_2 around 20 mg/L) was carried out for all the wines, in order to avoid microbial growth.

The free SO_2 amount tended to progressively decrease at a rate even higher than the total SO_2 content. In aged wines, this may be due to the progressive accumulation of acetaldehyde coming from the oxidation of ethanol, which strongly binds free SO_2.[31]

It can be noted that, if compared with oak, in cherry aged wines the free SO_2 amount was the lowest, in accordance with the highest content of acetaldehyde of those samples (Table 1). To try to explain such a finding, one may consider that the coupled oxidation of ethanol could be promoted to various extents by phenolics as a function of their degree of oxidizability.[15] Vivas and Glories[32] found that, if compared to (+)-catechin, oak

Table 1. Evolution of the Enological Parameters in Initial Wine and after 1, 2, and 4 Months of Aging in Wood Barrels[a]

		1 month				2 months				4 months			
	wine	steel	French oak (225 L)	cherry wood (225 L)	cherry wood (1000 L)	steel	French oak (225 L)	cherry wood (225 L)	cherry wood (1000L)	steel	French oak (225 L)	cherry wood (225 L)	cherry wood (1000 L)
pH	3.43 ± 0.01	3.43 ± 0.01	3.43 ± 0.00	3.44 ± 0.01	3.44 ± 0.01	3.41 ± 0.01	3.42 ± 0.01	3.44 ± 0.01	3.43 ± 0.01	3.42 ± 0.01	3.43 ± 0.00	3.47 ± 0.00	3.46 ± 0.01
volatile acidity (g/L)[b]	0.47 ± 0.04	0.48 ± 0.03	0.48 ± 0.04	0.48 ± 0.03	0.48 ± 0.04	0.46 ± 0.05	0.50 ± 0.03	0.48 ± 0.02	0.48 ± 0.04	0.48 ± 0.03	0.51 ± 0.04	0.52 ± 0.05	0.50 ± 0.03
titratable acidity (g/L)[c]	5.16 ± 0.00	5.16 ± 0.10	5.22 ± 0.01	5.20 ± 0.01	5.19 ± 0.11	5.24 ± 0.10	5.35 ± 0.01	5.25 ± 0.01	5.24 ± 0.1	5.20 ± 0.1	5.34 ± 0.0	5.21 ± 0.0	5.19 ± 0.1
reducing sugars (g/l)	1.80 ± 0.10	1.80 ± 0.10	1.65 ± 0.07	1.80 ± 0.10	1.80 ± 0.10	1.80 ± 0.10	1.75 ± 0.07	1.8 ± 0.00	1.80 ± 0.0	1.80 ± 0.1	1.80 ± 0.0	1.80 ± 0.0	1.80 ± 0.0
alcohol (v/v%)	13.19 ± 0.02 a	13.19 ± 0.01 a	13.22 ± 0.02 a	13.24 ± 0.01 a	13.20 ± 0.11 a	13.18 ± 0.01 a	13.21 ± 0.01 a	13.14 ± 0.01 ab	13.12 ± 0.02 ab	13.13 ± 0.00 ab	13.16 ± 0.01 ab	13.08 ± 0.00 b	13.10 ± 0.02 ab
total SO_2 (mg/L)[f]	66.0 ± 0.5 a	63.0 ± 1.7 a	59.5 ± 0.7 ab	58.0 ± 1.4 b	60.0 ± 1.0 ab	58.0 ± 0.6 b	47.0 ± 0.0 cd	44.0 ± 0.0 d	50.0 ± 1.5 c	52.0 ± 1.6 bc	57.0 ± 1.4 b	58.0 ± 1.4 b	62.0 ± 1.5 a
free SO_2 (mg/L)	25.0 ± 0.4 a	24.0 ± 0.9 a	21.5 ± 0.7 b	12.0 ± 0.5 d	13.0 ± 0.4 d	23.0 ± 0.4 ab	14.0 ± 0.7 cd	8.0 ± 0.1 e	9.0 ± 0.2 de	21.0 ± 0.3 b	16.5 ± 0.7 c	7.5 ± 0.7 e	9.0 ± 0.2 de
acetaldehyde	8.10 ± 0.2 f	9.12 ± 0.6 f	10.4 ± 0.9 ef	69.0 ± 4.2 b	43.7 ± 3.2 d	12.3 ± 1.1 e	38.3 ± 5.1 d	82.6 ± 6.9 b	59.5 ± 4.2 c	13.0 ± 1.3 e	41.3 ± 5.3 d	97.6 ± 7.3 a	64.7 ± 4.9 c
tannins (g/L)[d]	3.34 ± 0.01 f	3.40 ± 0.01 e	3.49 ± 0.04 d	3.62 ± 0.01 c	3.55 ± 0.02 d	3.43 ± 0.02 e	3.58 ± 0.04 cd	3.95 ± 0.03 a	3.63 ± 0.02 c	3.50 ± 0.04 d	3.61 ± 0.03 c	4.04 ± 0.02 a	3.76 ± 0.03 b
total phenolics (mg/L)[e]	2390 ± 23 bc	2376 ± 26 bc	2408 ± 31 b	2403 ± 18 b	2385 ± 20 bc	2350 ± 18 c	2380 ± 15 bc	2420 ± 28 b	2420 ± 25 b	2380 ± 16 bc	2390 ± 14 bc	2425 ± 7 b	2480 ± 22 a

[a] In the same row, different letters flag significant differences for $p < 0.05$. [b] Expresses as acetic acid. [c] Expressed as tartaric acid. [d] Expressed as cyanidin. [e] Expressed as gallic acid. [f] At the end of the second month, the total SO_2 level was brought to 60 mg/L

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Table 2. Changes in Native Anthocyanins in the Initial Wine and in the Samples at the End of Ageing[a]

			4 months			
anthocyanins		wine	steel	French oak (225 L)	cherry wood (225 L)	cherry wood (1000 L)
glucosydes	(mg/L)	221.0 ± 7.4 a	203.5 ± 6.6 b	172.7 ± 6.1 c	49.1 ± 3.7 e	95.3 ± 3.3 d
	(%)	86.1 ± 0.4 d	86.2 ± 0.4 d	87.0 ± 0.2 c	92.1 ± 0.6 a	90.5 ± 0.4 b
acetyl glucosydes	(mg/L)	26.0 ± 1.5 a	23.0 ± 1.2 b	19.4 ± 2.4 c	3.7 ± 0.1 e	10.4 ± 1.3 d
	(%)	10.1 ± 0.3 a	10.0 ± 0.2 a	9.8 ± 0.1 a	6.9 ± 0.2 c	7.6 ± 0.1 b
p-coumaroyl glucosydes	(mg/L)	9.8 ± 1.0 a	8.9 ± 1.2 a	6.3 ± 0.8 b	0.5 ± 0.3 d	3.2 ± 0.6 c
	(%)	3.8 ± 0.0 a	3.8 ± 0.0 a	3.2 ± 0.0 b	1.0 ± 0.0 d	1.9 ± 0.0 c
Sum native anthocyanins	(mg/L)	257.0 ± 8.8 a	235.0 ± 7.4 b	198.4 ± 9.3 c	53.3 ± 2.5 e	109.1 ± 4.1 d

[a] In the same row, different letters flag significant differences for $p < 0.05$.

Table 3. Pigment Derivatives Amount (mg/L) in the Initial Wine and in the Samples at the End of the Ageing[a]

		4 months			
compound[b]	initial wine	steel	French oak (225 L)	cherry wood (225 L)	cherry wood (1000 L)
		Direct Condensation Adducts			
Pn-3-glc(epi)catechin	0.3 ± 0.0 b	0.4 ± 0.0 a	0.3 ± 0.0 b	0.1 ± 0.0 d	0.2 ± 0.01 c
Mv-3-glc(epi)catechin	1.2 ± 0.1 b	1.4 ± 0.1 ab	1.6 ± 0.2 a	0.8 ± 0.1 c	1.1 ± 0.1 b
sum direct condensation adducts	***1.5 ± 0.1*** ab	***1.7 ± 0.1*** a	***1.9 ± 0.1*** a	***0.9 ± 0.1*** c	***1.4 ± 0.1*** b
		A-Type Vitisins			
A-type vitisin of Df-3-glc	0.2 ± 0.0 b	0.2 ± 0.0 b	0.2 ± 0.0 b	0.4 ± 0.0 a	0.2 ± 0.0 b
A-type vitisin of Pt-3-glc	1.4 ± 0.1 b	1.5 ± 0.2 ab	1.7 ± 0.1 a	1.3 ± 0.1 b	1.3 ± 0.1 b
A-type vitisin of Pn-3-glc	0.2 ± 0.0 b	0.2 ± 0.0 ab	0.2 ± 0.0 ab	0.2 ± 0.0 a	0.2 ± 0.0 a
vitisin A	2.8 ± 0.2 a	2.7 ± 0.2 a	2.7 ± 0.2 a	3.7 ± 0.1 b	3.3 ± 0.3 b
A-type vitisin of Mv-3-coumaroylglc	0.2 ± 0.0 c	0.2 ± 0.0 c	0.2 ± 0.0 b	0.2 ± 0.0 a	0.2 ± 0.0 b
sum A-type derivatives	***4.7 ± 0.3*** b	***4.8 ± 0.4*** b	***5.0 ± 0.4*** b	***5.7 ± 0.2*** a	***5.2 ± 0.4*** ab
		B-type vitisins			
B-type vitisin of Pt-3-glc	n.d.	n.d.	n.d.	0.6 ± 0.1 a	0.3 ± 0.0 b
vitisin B	0.6 ± 0.0 d	0.5 ± 0.0 e	0.7 ± 0.1 c	3.0 ± 0.1 a	1.6 ± 0.1 b
B-type vitisin of Mv-3-coumaroylglc	n.d.	0.1 ± 0.0 c	0.2 ± 0.0b	0.8 ± 0.0 a	0.8 ± 0.0 a
sum B-type derivatives	***0.6 ± 0.0*** d	***0.6 ± 0.1*** d	***0.9 ± 0.1*** c	***4.4 ± 0.3*** a	***2.7 ± 0.2*** b
		Ethyl-Bridged Adducts			
Mv-3-glc-ethyl(epi)catechin isomer	0.9 ± 0.1 c	0.5 ± 0.0 e	0.7 ± 0.0 d	1.7 ± 0.1 a	1.2 ± 0.1 b
Mv-3-glc-ethyl(epi)catechin isomer	1.3 ± 0.1 c	1.3 ± 0.1 c	1.4 ± 0.1 c	2.8 ± 0.2 a	2.3 ± 0.1b
Mv-3-glc-ethyl(epi)catechin isomer	2.1 ± 0.1 b	2.1 ± 0.1 b	2.4 ± 0.1 b	4.0 ± 0.1 a	4.2 ± 0.1+
sum ethyl-bridged adducts	***4.2 ± 0.3*** bc	***3.8 ± 0.2*** c	***4.4 ± 0.2*** b	***8.5 ± 0.5*** a	***7.7 ± 0.2*** a
		Vynylphenol Adducts			
pinotin A	0.2 ± 0.0 c	0.2 ± 0.0 d	0.3 ± 0.0 c	0.5 ± 0.0 a	0.3 ± 0.0 b
Mv-3-glc-4-vinylphenol	0.4 ± 0.02	0.4 ± 0.0 a	0.4 ± 0.0 a	0.4 ± 0.0 a	0.4 ± 0.0 a
Mv-3-acetylglc-4-vinylphenol	n.d.	0.2 ± 0.0 b	0.2 ± 0.0 b	0.2 ± 0.0 a	0.3 ± 0.0 a
sum vinylphenol adducts	***0.6 ± 0.1*** c	***0.7 ± 0.1*** c	***0.8 ± 0.0*** b	***1.1 ± 0.0a***	***0.9 ± 0.1*** a
sum adducts and derivatives	***11.6 ± 0.8*** d	***11.6 ± 0.6*** d	***13.0 ± 0.9*** c	***20.6 ± 1.0*** a	***17.9 ± 1.0*** b

[a] In the same row, different letters flag significant differences for $p < 0.05$. [b] Dp, delphinidin; Pt, petunidin; Pn, peonidin; Mv, malvidin; Glc, glucosyde; coumaroylglc, (6″-(*p*-coumaroyl)glucoside); A-type vitisin, pyruvic acid adduct; B-type vitisin, acetaldehyde adduct; vitisin A, pyruvic acid adduct of Mv-3-glc; vitisin B, acetaldehyde adduct of Mv-3-glc; pinotin A, Mv-3-glc-4-vinylcatechol.

wood ellagitannins induce a larger production of acetaldehyde owing to their higher oxidizing power. However, phenolics extracts from charred cherry wood does not resemble that of oak, being composed exclusively of catechins, flavonols, and other flavanones.[33] The influence of these latter compounds on acetaldehyde formation is largely unknown even if a relevant degree of oxidizability of not toasted cherry wood extracts has been already reported.[34] Undoubtedly, a confirmation of the importance of the volumes involved could be found by looking at the samples aged in the 1000 L cherry barrels, where the reduced wood surface-to-volume of wine ratio has limited the total amount of DO and/or the effects of phenolics cession to the

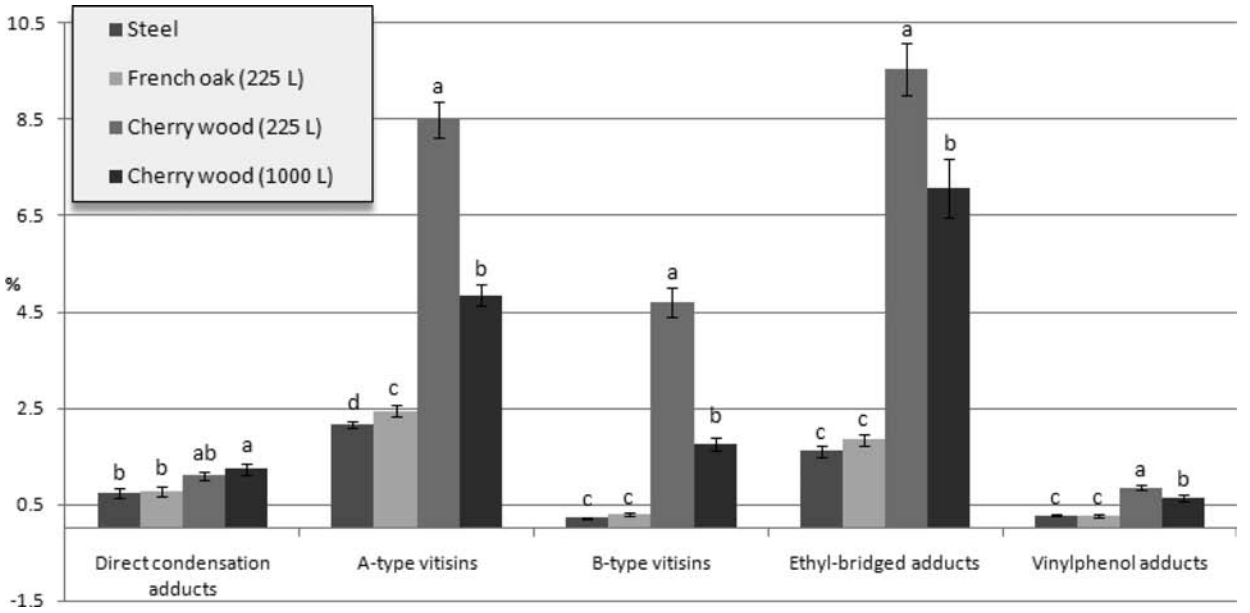

Figure 2. Percentage contribution of derivatives on the total amount of pigments in the wines after four months of aging (SD is given at the top of the bars).

wine and resulted in intermediate amounts of acetaldehyde, significantly lower than that found in the smaller 225 L cherry barrels.

The total content of phenolics evolved in a similar way in the four containers, with little or no changes during the storage time. This result is in contrast with the findings of De Rosso et al.,[17] who reported a significant decrease of polyphenols in cherry-aged samples after the first 3 months of storage with respect to oak and all the other species used in that investigation. The explanation of these differences are unclear but may reside in the different methods used to quantify such an index (Folin–Ciocalteau assay and UV absorption at 280 nm in the present and in that study, respectively).

With time, the amount of tannins increased in all the samples (Table 1). It has been reported that during the first months of storage, polymerized flavanol derivatives (especially trimers and tetramers) increase their content at the expense of monomers and dimers, mainly due to either direct or ethylidene-bridged condensation phenomena.[16] Furthermore, for wines aged in wood, polyphenols which are leaked from the cask can take part in these processes, augmenting the final tannin content. The presence of condensed tannins in cherry wood, together with the high content in acetaldehyde, hence, may account for the higher tannin values of wines aged in cherry casks, despite the weak percentage of extractable phenolics of such a wood when compared with that of uncharred oak or chestnut (25, 65, and 66%, respectively).[34]

Pigment Composition of Wines. As stated in the Materials and Methods section, since the aim of this work was devoted to pigment composition, we decided to stop the aging once the pigment amount (as determined by HPLC) of any one of the studied samples had reached a level such that the correct integration of acylated anthocyanins would become not consistently reliable. Table 2 shows that after 4 months of aging, the native anthocyanin amount significantly dropped in all the samples, and in wood aged wines, their lowering was more pronounced due to the augmented magnitude of oxidation and condensation phenomena.[35,36] If compared with oak, cherry wood favored the fastest diminution of native anthocyanins in both 225 L and 1000 L barrels. However, the larger 1000 L casks had a total pigment amount nearly double with respect to the 225 L cherry barriques, further corroborating the assumption that the volume-to-surface ratio plays a significant role in the mechanisms that govern pigment decrease.

The qualitative composition of native pigments also changed with time (Table 2). At the fourth month, wines aged in cherry wood had the lowest percentage of acylated anthocyanins if compared to the ones stored in either the stainless steel or oak containers. For cherry aged wines, this result is in accordance with the findings of De Rosso et al.[17] who suggested the cause could be some rearrangements and/or oxidation reactions. In fact, the changes in anthocyanin percentages demonstrate the higher susceptibility of acylated pigments to undergo losses during aging not only as the consequence of their oxidation but also probably because of their hydrolysis and conversion to glucosides.[37] Once again, the 225 L cherry barriques provoked the strongest variation of the pigment profile which, at the fourth month, was almost devoid of *p*-coumaroyl glucosides (Table 2). This suggests that, in the case of long aging times, wines kept in cherry wood may notably diverge from the anthocyanin fingerprint of the initial wine, especially when cultivars with significant amounts of constitutive acylated anthocyanins are concerned.

It is well accepted that, during winemaking, a series of condensation reactions between native anthocyanins and wine components take place.[2−5] These phenomena lead to new classes of pigments which, during wood aging, could differently evolve and affect the chromatic features of wines.[6,8,9]

In Table 3, both the initial and final content in wines of these newly formed compounds are shown grouped as a function of their pigment family. Their identification and quantification was carried out according to the HPLC/DAD and HPLC/MS methods previously published.[9]

In general, in wood aged wines, the total amounts of adducts and derivatives increased with time. Cherry wood promoted the highest amount of adduct formation compared with that of oak. In terms of percentages, at the beginning of storage, derivatives represented about 4.5% of native anthocyanins (see initial wine

Table 4. Evolution of Color Features of the Wines during Wood Ageing[a]

		2 months				4 months			
	wine	steel	French oak (225 L)	cherry wood (225 L)	cherry wood (1000 L)	steel	French oak (225 L)	cherry wood (225 L)	cherry wood (1000 L)
color intensity (A.U.)*	8.48 ± 0.01 f	8.47 ± 0.02 f	8.63 ± 0.01 e	10.9 ± 0.01 c	10.45 ± 0.02 c	8.61 ± 0.04 e	9.07 ± 0.02 d	12.8 ± 0.05 a	12.13 ± 0.02 b
shade	0.74 ± 0.02 a	0.69 ± 0.01 b	0.69 ± 0.01 b	0.63 ± 0.01 c	0.61 ± 0.03 c	0.71 ± 0.03 ab	0.71 ± 0.05 ab	0.68 ± 0.01 b	0.67 ± 0.02 b
WC (A.U.)	5.67 ± 0.21 b	5.74 ± 0.36 b	5.77 ± 0.19 b	6.20 ± 0.41 a	6.00 ± 0.33 ab	5.00 ± 0.37 c	5.00 ± 0.25 c	3.74 ± 0.16 e	4.60 ± 0.12 d
WCP (A.U.)	17.92 ± 0.98 b	17.22 ± 0.01 bc	17.70 ± 0.01 b	20.50 ± 0.01 a	20.24 ± 0.03 ab	17.00 ± 0.03 c	17.48 ± 0.05 bc	15.06 ± 0.01 c	17.93 ± 0.02 b
copigmentation (%)	9.8 ± 0.2 a	8.5 ± 0.3 b	8.6 ± 0.2 b	0.4 ± 0.0 e	0.8 ± 0.0 e	2.8 ± 0.1 c	0.9 ± 0.2 d	0.0 ± 0.0 e	0.0 ± 0.0 e
CAW (%)	45.1 ± 0.3 e	50.8 ± 0.2 d	51.9 ± 0.3 d	65.4 ± 0.3 b	57.3 ± 0.3 c	56.2 ± 0.5 c	58.4 ± 0.3 c	86.9 ± 0.6 a	85.2 ± 0.5 a
CDD (%)	23.3 ± 0.1[9]	23.0 ± 0.2 g	29.06 ± 1.0 f	63.43 ± 0.2 c	50.68 ± 0.2 d	25.84 ± 1.3 fg	38.91 ± 0.2 e	81.0 ± 0.8 a	67.75 ± 0.3 b

[a] In the same row, different letters flag significant differences for $p < 0.05$. *Absorbance Units.

in Tables 2 and 3), while, after 4 months, their percentages spanned between 6.5% and 38%, respectively, for oak and cherry 225 L casks. Furthermore, whatever the sample considered, A-type vitisins and ethyl-bridged adducts appeared to be the prevailing pigment derivatives (corresponding to about 70% to 75% of the sum of derivatives), in accordance with the results of other authors[37,38] for wines at 3 or 4 months of wood aging.

Cano-Lopez et al.[38] and Alcalde-Eon et al.[39] established that oxygen plays a main role in the reactions preceding the formation of both the pyranoanthocyanins and the ethyl-bridged adducts. De facto, on the basis of our results, these two classes of compounds were found to increase following the order 225 L cherry cask > 1000 L cherry barrel > 225 L oak cask ≥ stainless steel tank, hence suggesting a possible ranking in the oxidative environment to which the wine was subjected. It is worth mentioning, however, that ethyl-bridged adducts are expected to decrease after a few months due to their weak chemical stability.[38,39] They release ethyl-catechin residues, which, in turn, may give rise to more condensed anthocyanin-flavanol polymers.

In cherry aged wines, B-type vitisins were a further main family of derivatives (Table 3), probably stemming from the high acetaldehyde content of those wines (Table 1).

Direct condensation adducts seemed to be the only group of derivatives whose content was higher in wines stored in stainless steel or aged in oak casks (Table 3). This fact may suggest a greater stability of directs adducts in environments with relatively slow oxygen dissolution kinetics and confirms other published results in which nonoxygenated wines had increased amounts of these pigments with respect to both oxygenated and wood aged wines.[38]

Taking into account their specific color features, derivatives may well influence the perceived sensory attributes of wines if present in considerable amounts with respect to the total coloring matter of the samples. In order to further highlight this aspect, in Figure 2 is shown the percentage contribution of each group of derivatives over the total amount of pigments (sum of native anthocyanins and derivatives) after four months of aging. Also because of their low content in monomeric anthocyanins, cherry aged samples appeared to be markedly characterized by the high percentages of ethyl-bridged adducts and both the A- and B-type vitisins (about 23% of total pigment amount in 225 L cherry wood aged wines). Once again, the use of the larger 1000 L casks seemed to mitigate the effect of the cherry wood and produced wines with lower percentages of derivatives, whose values were intermediate between those from 225 L oak and those from cherry barriques.

The aging in oak barriques changed the pigment composition to a lower extent and, when compared with stainless steel storage (Figure 2), changed little, but by significant increments in A-type vitisins.

Evolution of Wine Color. In Table 4, the evolution of wine chromatic parameters after two and four months of storage are reported.

For all the samples, color intensity increased during the aging period. This result agrees well with other published data[35,38] and has been linked to the progressive formation of derivatives, some of which feature high molar absorption and chromatic stability.[10] The wines stored in cherry wood consistently had the highest values of color intensity with respect to that of the other samples. This could be somewhat surprising when the total amount of pigments of such samples (which showed the lowest content of anthocyanins) is taken into account (see Table 2). However, in a

previously published work dealing with the influence of pigments on the color of carbonic macerated wines,[9] we found the color intensity to be strongly correlated with both A- and B-type vitisins and ethyl-bridged adducts, while no correlation was found with native anthocyanins. Cherry aged wines had, in fact, the highest amount (and the highest percentage) of pyranoanthocyanins and ethyl-bridged adducts, and the relationship of these compounds with color intensity, also confirmed by Boido et al.,[37] further suggests the great role played by pigment derivatives in the chromatic features of aged wines.

Evolution of color shade describes the tendency of wine to brownness. Data in Table 4 show that after 2 months, not only did the cherry aged wines have a deeper color but also a somewhat redder nuance than the other wines. Two further months of aging reduced such differences in color shade, and all the wines had a similar nuance independently from the aging system.

It is widely accepted that larger pigmented polymers, produced during the wood contact time, also contribute to a large extent to the perceived color of final wines. Because of its heterogeneity, this class of compounds could hardly be evaluated by conventional HPLC and is often measured by inference, using spectrophotometric techniques.[40]

In Table 4, apart from the CAW index, which is a spectrophotometric estimation of the color due to the nonbleachable pigments,[36] we introduced a further index (CDD), obtained by combining both HPLC and photometric data. This parameter would be intended as an additional way to estimate the contribution of adducts and polymers to the wine color. As far as these two indexes are concerned, it clearly appears that nonbleachable (e.g., derivatives and/or polymeric) pigments increased during the aging time in all the wines, up to exceed the color given by native monomeric anthocyanins. As already reported,[9] compared to CDD, the CAW index seems to overestimate the contribution to the color of derivatives and polymers. However, both the two indexes reveal that at the fourth month, more than 80% of the color of wines aged in 225 L cherry barrels is due to derivatives and polymers. This fact furnishes a further explanation for the high color density of those samples despite their low content in anthocyanins. On the basis of the CDD index, in French oak barriques, derivatives contributed to wine color to a lower extent. Their percentages were, however, significantly higher than those obtained for stainless steel and are expected to increase in the case of longer aging periods.

The degree of copigmentation of the initial wine was quite low (Table 4). This may depend on the low cofactor (e.g., flavonols and phenolic acids)-to-pigment ratio of the wine.[28] However, aging further reduces the contribution of copigmentation to wine color because of the formation of polymeric pigments unable to establish hydrophobic interaction with copigments.[28] Our data confirm this decrease for all the samples studied and show that in wines aged in cherry casks, copigmentation is virtually absent since the first months of aging, probably as a consequence of the above-mentioned enhanced formation of polymeric pigments and/or diminution in flavonols and cinnamic acids which are considered the main copigmentation factors in wines.

In summary, the use of cherry casks appeared to deeply influence the color and the pigment composition of red wines. Despite the increased color density of final wines, if compared to oak, cherry wood provoked the fastest evolution of native anthocyanins, leading to a steady reduction of monomeric anthocyanins and enhanced accumulation of derivatives and polymers. Anthocyanin profile of final wines was significantly changed by wood aging, the cherry casks driving to an almost complete disappearance of the *p*-coumaroylated pigments. Further studies are necessary to understand the role played by either wood porosity or phenolic composition of cherry wood in promoting the above-mentioned phenomena. In order to better manage wine color evolution while maintaining adequate amounts of native anthocyanins, short aging times should be preferred when cherry barriques are to be used. Alternatively, the use of larger casks (e.g., 1000 L) with a reduced wood surface-to-wine volume ratio could be an effective option.

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