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# Effect of Oenotannin Addition on the Composition of Sangiovese Wines from Grapes with Different Characteristics

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**Abstract:** Different types of oenotannins were added to *Vitis vinifera* cv. Sangiovese grapes of various composition and the resulting wine color parameters were measured, including intensity, hue, total phenol index, monomer anthocyanin content, and colored polymeric pigment content. Oenotannins were also tested during pre- and postfermentation on grapes harvested in 2003 from two growing areas. Grapes from the 2004 harvest were tested at different ripeness using only oenotannins exhibiting the best wine color stabilization in 2003. The response of different oenotannins varied according to grape characteristics, with gallnut and grape seed tannins most reliably stabilizing and increasing color intensity even after six months of storage. The same tannins positively affected wine color stabilization, an effect further enhanced in wine produced from less ripe grapes. Timing of oenotannin addition and grape characteristics had a significant effect on color intensity and stabilization. The estimation of grape phenolic maturity may allow for improved wine color characteristics by tailoring the use of oenotannins.

**Key words:** oenotannins, prefermentation addition, postfermentation addition, Sangiovese grape, wine color indices

Current winemaking methods derive, in part, from traditional schemes and from innovations that have resulted from particular needs, such as management and control of wine during fermentation, management of oxygen during wine aging, and stabilization treatments. In winemaking, the use of enological tannin (oenotannin) has received renewed attention (Parker et al. 2007, Main and Morris 2007, Álvarez et al. 2009). Oenotannins are substances of plant origin, derived from several botanical species (Bertrand et al. 2000, Vivas et al. 2004, Laghi et al. 2010). These products are generally classified according to their origin into two groups: hydrolyzable tannins, derived mainly from oaks or other plant species, and condensed tannins, mainly from grapes. Hydrolyzable tannins include glucosides, either from gallic acid (gallotannins from tara, myrabolan fruit, gallnuts) or from ellagic acid (ellagitannins from oak, chestnut). Condensed tannins (grape, quebracho wood) are composed of flavan-3-ol monomer subunits, such as catechin, epicatechin, and their gallates. Initially proposed as coadjuvants to prevent the wine proteic instability and officially authorized by the International Oenological Codex (OIV

2009), oenotannins have recently been introduced into white and red winemaking. Some of the claimed positive effects of oenotannins include wine color stabilization, improved wine structure, control of laccase activity, and elimination of reductive odors (Crespy 2003a). The mechanism of action is different depending on the nature of the oenotannin. Condensed tannins (proanthocyanidins) can combine with anthocyanins, directly or by acetaldehyde-mediated reactions, and stabilize wine color (Somers and Wescombe 1987, Fulcrand et al. 2006). Hydrolyzable tannins have an important role in balancing the reactions of color stabilization of red wines (Saucier et al. 2006). In particular, ellagitannins can function as oxidation regulators, quickly reacting dissolved oxygen and facilitating the hydroperoxidation of wine constituents. This reaction induces tannin/anthocyanin condensation via acetaldehyde, favoring stabilization and deepening the crimson color, while the limited oxidation of wine phenolic compounds prevents the development of brick-yellow color. The role of hydrolyzed tannins in providing improvement in wine chromatic characteristics has led to the speculation that these tannins cannot react directly with anthocyanin. A recent study conducted in mildly acidic (pH 3–4) hydroalcoholic wine model solution has shown that hydrolyzed tannins (vescalagin) can react with red-colored grape-derivate pigment (oenin) that furnishes a novel purple-colored anthocyano-ellagitannin hybrid pigment (Chassaing et al. 2010). In addition, the structure of wine tannin can change, reducing wine astringency because of higher polymerization (Vivas and Glories 1996).

While many winemakers have practical experience with oenotannin addition, there have been few replicated research studies of the effects. Tannin suppliers report color enhancement, oxidative protection, and flavor and mouthfeel improvements, although there are differing opinions regarding the

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Supplemental data is freely available with the online version of this article.

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benefits of different products. Several reports by French researchers, appearing in wine industry trade publications, provide some evidence of the benefits to wine color from tannin preparations added during red wine vinification. Treatment of rosé wines with grape-derived tannins (at 1000–2000 mg/L) did not improve retention of color intensity (Crespy 2003a), while there have been reported benefits to color intensity in Syrah (Lurton et al. 2002) and various red and rosé wines (Crespy 2002). An increase in red color soon after tannin addition has been attributed to copigmentation effects (Bertrand et al. 2000). Seed supplementation (twice the typical amount) increased the wine color density of wines made from Merlot, but had little effect on wines made from Frankinja (Revilla et al. 1998). In a similar study, wines made with supplementary seed additions (double or triple the typical amount) to must from Cabernet Sauvignon, Merlot, Pinot noir, Vranac, Garnacha, and Tempranillo resulted in variable improvement in wine color compared to the same wine with no addition, but generally seed additions were reported to increase color density (Kovac et al. 1995). The effects of exogenous tannin addition on the properties of a red wine made from *Vitis vinifera* cv. Shiraz were investigated in a study performed with pre- and postfermentation additions of a commercial grape seed-derived oenotannin product (Parker et al. 2007). The influence of the added oenotannin on wine tannin concentration, pigmented polymer concentration, and color parameters was monitored throughout the winemaking process and for up to two years of postbottling storage. After one year of storage, the in-mouth sensory attributes of the wines were characterized using descriptive analysis. No significant differences in wine color properties and pigment profiles were found between treatments. For the type of red wine and oenotannin studied, tannin addition did not affect wine color properties and had only a minor effect on perceived astringency. The authors suggested that this effect was probably attributable to a natural polyphenol richness in the grapes and that phenolic maturity should determine the requirement for physicochemical treatments in the winemaking process. Several studies confirmed the effect of the degree of grape ripening on wine color and on the level of flavanol and anthocyanin compounds (Pérez-Magariño and González-San José 2004, Ortega-Regules et al. 2008), which are responsible for the wine color attributes.

In general, studies on oenotannins emphasize that they should be used with great care, because they may not always improve wine characteristics; indeed, wines may lose their equilibrium, an effect more pronounced when a hydrolyzable tannin was used (Obradovic 2006). The addition of selectively extracted grape-derived tannins may compensate for tannin deficiency in grapes. Polyphenolic imbalance can be linked to either grape variety or vintage and expressed by a deficiency or an excess of tannins or anthocyanins. This imbalance may produce wines with harsh, bitter, or unstructured tannins, wines with weak, unstable color, or wines predisposed to oxidative characters. The tailored use of oenotannins should be adopted from the beginning of the alcoholic fermentation and maintained throughout the aging process.

Research on the effect of tannin applications has been conducted on few grape varieties, including Shiraz (Lampereur et al. 2002, Parker et al. 2007), Cabernet Sauvignon (Crespy 2003b), Merlot (Crespy 2002, 2003b), Grenache (Crespy 2002), and Gamay (Lampereur et al. 2002). However, no information is available for Sangiovese, an important red grape variety in Italy and particularly in Tuscany, where it is the base for Chianti and Brunello wines. Sangiovese wine (very similar to Pinot noir) can have problems with color stability, and the grapes are rich in anthocyanins, such as cyanidin-3-glucoside and peonidin-3-glucoside, and in unstable and oxidizable phenols (Di Stefano et al. 1994).

The aim of this work was to verify the interaction effects between *V. vinifera* cv. Sangiovese grapes of different phenolic composition and different kinds of oenotannin addition on wine color stabilization. Several parameters were used to describe the wine color, including color intensity, hue, total phenol index, monomeric anthocyanin content, and colored polymeric pigment content. The effect of using oenotannins at different points in the winemaking process was also examined.

## Materials and Methods

**Chemicals and oenotannins.** Acetonitrile and *o*-phosphoric acid were HPLC grade (Panreac, Barcelona, Spain). (+)-4-Methylcatechol and benzyl mercaptan were from Sigma-Aldrich (Milan, Italy). Gallic acid and Folin–Ciocalteu reagent were from Fluka (Bush, Switzerland). Cyanidin chloride and malvidin-3-glucoside hydrochloride were HPLC grade (Extrasynthèse, Genay, France). The other chemicals were of high purity and were purchased from Sigma-Aldrich.

Six commercial oenotannins were used: grape skin (S) (Grap'tan S; FERCO, St. Montan, France), grape seed (V) (Grap'tan V; FERCO), grape seed (W) (Grap'tan E; FERCO), chestnut (C) (Tannoplus, EverIntec Company, Verona, Italy), oak (O) (Tanstructure; EverIntec), and gallnut (G) (Tanncolor; EverIntec).

**Instrumentation.** HPLC analysis was carried out on a PerkinElmer Series 200 LC equipped with an Autosampler and Diode-Array Detector (PerkinElmer, Waltham, MA). UV/visible absorbance readings were measured on a spectrophotometer (Lambda 35 UV/Vis; PerkinElmer).

**Grape samples.** Sangiovese grapes from the 2003 and 2004 seasons were hand-harvested from three vineyards in the Chianti Classico area (Tuscany, Italy). The vineyard growing area of grape group *a* and group *c* was characterized by rocky soils rich in clay content, while that of group *b* had rich deposits of alluvial material which represented a greater deep-rooting potential. In 2003, the *a* and *b* grapes were harvested the same day (20 Sept 2003) at 24.6 and 24.4 Brix, respectively. In 2004, the grapes of group *c* were harvested at three different ripeness stages, designated 1, 2, and 3, which were 20 Sept, 5 Oct, and 19 Oct at 20.2, 22.6, and 25.0 Brix, respectively (Table 1).

**Winemaking conditions.** All wines were made in duplicate batches in 25-L capacity stainless-steel vats. After crushing and destemming, grape must received an addition of 50 mg/L SO<sub>2</sub>, and after 1 hr the must was inoculated with

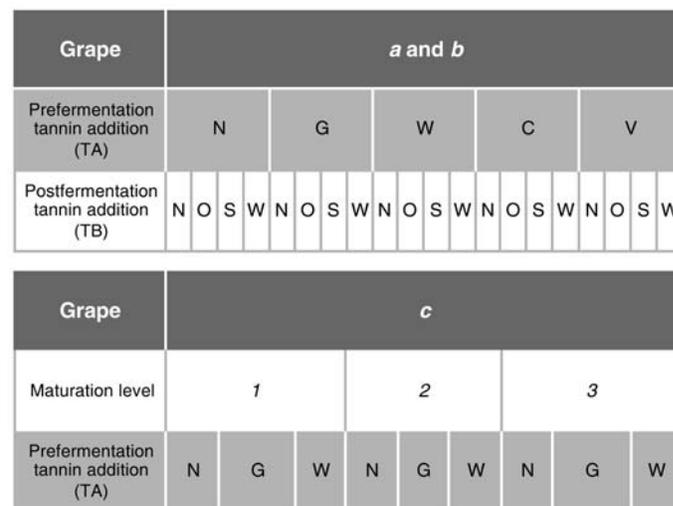
0.2 g/L rehydrated active dried yeast (GRE, Laffort, Bordeaux, France), 0.3 g/L yeast nutrient (Biocibus Rossi; EverIntec), and 0.025 g/L maceration enzyme (Biozim P Rossi; EverIntec). Fermentors were stored at 25°C for 14 days and the caps were mixed into the fermenting wines twice daily. On the second day of fermentation, 0.3 g/L yeast nutrient (Biocibus Rossi; EverIntec) was added. Alcoholic fermentation was completed after 10 days when the residual sugars were less than 2 g/L. The wines were maintained for another four days with the pomace, with one pushing down daily, to allow postfermentation maceration. After 14 days of maceration, all wines were pressed with a pneumatic press and press wine up to 2 bar added back to free-run wine. Wine were transferred to 15-L glass carboys, inoculated with 0.02 g/L rehydrated culture of *Oenococcus oeni* (ID; Lallemant, Montréal, Canada) and kept at 20°C until the end of malolactic fermentation. After malolactic fermentation, the wines from the 2003 vintage were racked, total SO<sub>2</sub> adjusted to 50 mg/L, and microoxygenated (2 mg/L/month) for 6 months at 18°C. At the end of microoxygenation the wines were racked, total bisulfite adjusted to 80 mg/L, bottled, and stored at 18°C before analysis. The 2004 wines after malolactic fermentation were racked, total SO<sub>2</sub> adjusted to 80 mg/L, bottled, and stored at 18°C before analysis.

**Oenotannin addition.** *Prefermentation.* Prior to fermentation, musts from grape groups *a* and *b* (2003 season) were divided in five duplicate lots. One assigned treatment received no tannin addition, while four oenotannin treatments were assigned to four duplicate must samples. A water solution (10 g/L) of each commercial oenotannin was added to must and wine to reach a concentration of 0.2 g/L, whereas the remaining lot with no tannin addition represented the control (Figure 1). Musts from group *c* (2004 season) were divided in three duplicate lots (1, 2, 3). One assigned treatment received no tannin addition, while two oenotannin treatments were assigned to two duplicate must samples.

*Postfermentation.* At the end of malolactic fermentation, 0.15 g/L of three oenotannins was added separately to a sample of each wine from groups *a* and *b*. Wines codes are reported in Figure 1.

**Oenotannin analysis.** Each oenotannin was prepared as a 1 g/L solution with a hydro-alcoholic buffer (12% ethanol, pH 3.2 with tartaric acid). The total phenol concentration for each oenotannin solution was estimated using Folin–Ciocalteu reagent and expressed as gallic acid equivalents. Quantification was based on the standard curve of 25, 50, 100, and 200 mg/L gallic acid prepared at the same time and conditions. In performing the measurements, 1 mL Folin–Ciocalteu reagent was added to a 50 µL aliquot of each sample. After stirring the solution for 5 min, 4 mL of a solution of sodium carbonate (10% w/w in water) was added and made to a final volume of 20 mL with deionized water. The mixture was allowed to stand for 90 min at room temperature and in the dark. The absorbance was then read at 760 nm using a 10 mm path-length quartz cell.

Total proanthocyanidins were determined according to an established method (Di Stefano et al. 1989). 100 µL Oenotannin hydro-alcoholic solution was added to 5.25 mL ethanol



**Figure 1** Schematic overview and identifying code of the wines produced (grape *a*, *b*, 2003 harvest; grape *c* (1, 2, 3), 2004 harvest; N, no tannin addition (control); G, gallnut tannin; W, grape seed tannin; C, chestnut tannin; V, grape seed tannin; O, oak tannin; S, grape skin tannin).

**Table 1** Composition of Sangiovese grapes at harvest, 2003 and 2004: mean values and least significant difference (LSD).

Harvest date	Grape group	pH	Titrateable acidity (g/L tartaric acid)	Sugar (Brix)	Extractable anthocyanins (ApH3.2, mg/L M3MG)	Total potential anthocyanins (ApH1.0, mg/L M3MG)	Cellular maturity index (EA%)	Phenolic richness (OD280 nm)	Seed maturity (%)	Skin tannins (DTpell)	Seed tannins (DTpép)	M3MG (mg/L) <sup>a,b</sup>	CPP (mg/L M3MG) <sup>a,b</sup>
<b>2003</b>													
20 Sept	<i>a</i>	3.54 c <sup>c</sup>	5.18 b	24.6 c	652 a	1197 a	44 c	84 c	69 e	26 a	57 c	75 a	11 a
20 Sept	<i>b</i>	3.60 d	5.32 c	24.4 c	1687 d	2737 d	37 a	127 d	46 d	68 c	56 c	183 d	16 c
<b>2004</b>													
20 Sept	<i>c1</i>	3.28 b	6.49 d	20.2 a	724 b,c	1308 b	43 b	40 a	27 b	30 b	11 a	81 b	12 a
5 Oct	<i>c2</i>	3.12 a	6.52 e	22.6 b	703 b	1298 b	46 d	38 a	25 a	26 a	10 a	82 b	12 a
19 Oct	<i>c3</i>	3.54 c	5.10 a	25.0 d	750 c	1510 c	49 e	48 b	36 c	31 b	18 b	95 c	14 b
<b>LSD<sup>c</sup></b>		0.01***	0.02***	2.14***	13.11***	28.77***	0.48***	0.86***	0.57***	0.52***	0.62***	2.44***	0.49***

<sup>a</sup>M3MG, total amount of free anthocyanins expressed as malvidin-3-monoglucoside; CPP, colored polymeric pigments content expressed as malvidin-3-monoglucoside.

<sup>b</sup>Determined by HPLC on the grape extracts (pH 3.2) for the determination of the phenolic maturity of grapes.

<sup>c</sup>Different letters within the same row indicate significant differences; for LSD, \*\*\* indicates significance at  $p \leq 0.001$ .

and 7.15 mL 12 N HCl containing 300 mg/L  $\text{FeSO}_4 \cdot x \cdot 7\text{H}_2\text{O}$  in a Pyrex tube in an ice bath. The tube was hermetically sealed and heated at 100°C for 1 hr in a water bath. After stirring, the visible spectrum (400–700 nm) was determined using a 10 mm path-length quartz cell. Results were expressed as mg cyanidin/g.

The mean degree of polymerization (mDP) of oenotannins (only the condensed tannins S, W, and V) was determined according to an established method (Vivas et al. 2004). The oenotannin solutions were treated to separate the monomeric phenol compounds before acid-catalyzed depolymerization. The procedure for the phenol monomeric separation was the following: after a neutral C18 Sep-Pak cartridge 10 g (Waters Corp., Milford, MA) was preconditioned by 25 mL methanol and then water, oenotannin solution (3 g/L water) was passed through by a SPE vacuum device, which allowed eight samples to be handled simultaneously. The phenolic acids did not adsorb on the hydrophobic C18 stationary phase and were eliminated. The hydrophobic polyphenols were adsorbed onto the column and not eluted by water. Next, 20 mL 0.01 N  $\text{H}_2\text{SO}_4$  was added to the cartridge to acidify the matrix, and a solvent (acetonitrile:0.01 N  $\text{H}_2\text{SO}_4$ :water, 16:10:74) was used to elute catechins and flavonols. Finally, methanol (40 mL) was used to remove the polymeric fraction. The methanolic fraction was evaporated under reduced pressure at 40°C. The residual solid was reconstituted into methanol to a 1 g/L final concentration and 0.5 mL methanolic purified oenotannin solution was placed in a glass ampoule with an equal volume of reagent (5% solution of benzyl mercaptan in MeOH containing 1.7% HCl). After sealing the ampoule, the mixture was shaken and heated at 60°C for 10 min. 0.5 mL water containing 0.1 g/L 4-methylcatechol was added to the hydrolyzed solution to prevent the formation of asymmetric peaks and to improve chromatographic resolution. The solution obtained was analyzed directly by HPLC. A 250 x 4.6 mm LiChrospher RP-18 column (5  $\mu\text{m}$ ; Alltech, Milano, Italy) was used with a guard cartridge (10 x 4.6 mm) packed with the same materials. Both columns were maintained at 30°C. Prior to injection, samples were filtered at 0.22  $\mu\text{m}$ . The injection volume was 20  $\mu\text{L}$  and the sample was eluted with a flow rate of 1 mL/min by following gradient of solvent A (aqueous 5% (v/v) acetic acid) and solvent B (5% (v/v) methanol in acetic acid): from 30 to 100% solvent B in the first 35 min, held isocratic at 100% from 35 to 40 min, increased from 100% to 30% from 40 to 45 min. Chromatograms were acquired at 280 nm, recorded, and processed using Total Chrome Navigator software (PerkinElmer).

The mDP of oenotannins was calculated as the ratio between the total sum of released units (intermediate and terminal) and the sum of terminal released units.

Oenotannin preparations were also analyzed by HPLC by a previous method (Peng et al. 2002). Prior to injection, oenotannin extracts were filtered at 0.22  $\mu\text{m}$ . Injection volume was 20  $\mu\text{L}$  and the phenolics were eluted with a flow rate of 1 mL/min by the following gradient of solvent A (aqueous 1.5% (v/v)  $\text{H}_3\text{PO}_4$ ) and solvent B (20% (v/v) solvent A in  $\text{CH}_3\text{CN}$ ): from 8 to 27% solvent B in the first 55 min, held isocratic at

27% from 55 to 59 min, reduced from 27% to 70% from 59 to 64 min, held at 70% from 64 to 69 min, and increased to 8% from 70 to 76 min. Chromatograms were acquired at 280 nm, recorded, and processed using Total Chrome Navigator software (PerkinElmer).

**Grape analysis.** Commercial ripeness was measured by EU methods (Community Methods for the Analysis of Wines, Commission regulation 440/2003). Two hundred berries were pressed to separate juice. Sugar content (Brix), titratable acidity (g/L), and pH were measured after centrifugation of juice at 3000 rpm for 10 min.

Phenolic maturity was measured according to an established method (Saint-Criq et al. 1998). Two lots of 50 g mixture obtained from homogenization of 200 berries by an Ultra-Turrax (Staufen, Germany) high-speed at 11,500 rpm for 30 sec were put into two flasks. Fifty mL 0.1 N HCl solution at pH 1.0 was added to the first flask; 50 mL tartaric acid solution (i.e., 5 g tartaric acid in 20 mL 1 N NaOH, completed to 1 L vol. with distilled water) at pH 3.2 was added to the second flask. Flasks were stirred at room temperature for 4 hr, and samples were then centrifuged (10,000 rpm, 4°C, 10 min). The following parameters were determined by spectrophotometric analysis of the extracts. Phenolic richness (OD280 nm), related to phenolic compounds content in berries, was determined by absorbance at 280 nm of extracts at pH 3.2. Total potential anthocyanin (ApH1.0), expressed as mg/L malvidin-3-monoglucoside (M3MG), related to anthocyanin content in berries, was determined by absorbance at 520 nm of extracts at pH 1.0. Extractable anthocyanin (ApH3.2, mg/L M3MG), related to extractable anthocyanins at wine pH, was determined by absorbance at 520 nm of extracts at pH 3.2. Cellular maturity index (EA%) showed the ability of berries to release anthocyanins and was determined as:  $\text{EA} = (\text{ApH1} - \text{ApH3.2})/\text{ApH1} \times 100$ . The lower the EA index value, the higher the extractable potential of anthocyanins. Seed maturity index (MP%) showed the ability of seeds to release tannins and was determined as:  $\text{MP}\% = [\text{OD280nm} - (\text{ApH3.2}/1000) \times 40]/\text{OD280nm}$ . The higher the MP% index value, the higher the potential of extractable tannins from seeds. Skin tannin (DTpell) showed the ability of skin berries to release tannins and was determined as:  $\text{DTpell} = \text{EA}\% \times 40/1000$ . Seed tannin (DTpep) showed the ability of seeds to release tannins and was determined as:  $\text{DTpep} = \text{OD280nm} - \text{DTpell}$ .

Free anthocyanins and colored polymeric pigments (CPP), both expressed as mg/L M3MG, were determined by HPLC (Peng et al. 2002). Prior to injection, grape extracts at pH 3.2, obtained with the method to determine the phenolic maturity, were centrifuged (10,000 rpm, 4°C, 10 min), followed by addition of 1.5% formic acid and filtered at 0.22  $\mu\text{m}$ . Chromatograms were acquired at 520 nm. HPLC conditions were the same as reported for oenotannin analysis.

**Wine analysis.** Color intensity (CI) and wine hue (H) were both measured using a 1 mm path-length quartz cell and distilled water as a reference. CI was expressed as the sum of absorbances at 420 nm (A420), 520 nm (A520), and 620 nm (A620):  $\text{CI} = (\text{A420} + \text{A520} + \text{A620}) \times 10$ . Hue was expressed

as the ratio between absorbance at 420 nm (A420) and 520 nm (A520):  $H = A420/A520$ . Total phenol index (TPI) was measured as absorbance at 280 nm using a 10 mm path-length quartz cell and distilled water as a reference. Samples were diluted 1:100 with distilled water.

Monomer anthocyanin content (expressed as mg/L M3MG) and colored polymeric pigments (CPP) were determined by HPLC (Peng et al. 2002). Prior to injection, wines were centrifuged (10,000 rpm, 4°C, 10 min), followed by addition of 1.5% formic acid and filtered at 0.22 µm. Chromatograms were acquired at 520 nm. HPLC conditions were the same as for oenotannin analysis. (Full wine analysis data are presented in Supplemental Figure 1 and Supplemental Tables A–I.)

**Statistical analysis.** All analytical data were analyzed using Statgraphics Centurion (ver. XV, StatPoint Technologies, Warrenton, VA) with multifactor ANOVA considering grapes, tannins, timing of tannin addition, and replication as factors. Principal component analysis (PCA) and partial least squares analysis (PLS) were performed using Unscrambler (V9.1, CAMO Process AS, Oslo, Norway). The test set validation (Wold 1978) was used to test the number of significant principal components.

## Results

**Characterization of oenotannins.** To explain the effect of oenotannins on determining wine color stabilization, the different oenotannins used for the fermentation and maturation trials were characterized by UV-Vis spectroscopy and HPLC (Table 2). Total phenolic concentration ranged from 518 to 873 mg equivalent gallic acid/g tannin. Total proanthocyanidins were determined only on grape oenotannin products (S, V, W) and ranged from 629 to 789 mg equivalents cyanidin/g tannin. The mean degree of polymerization (mDP) for grape seed tannins was 2.63 and 4.07 for W and V oenotannin, respectively, and was 2.0 mDP for grape skin tannins.

HPLC was used to assay and profile phenolics and tannins, and six representative chromatograms of oenotannin hydroalcoholic buffer solutions were recorded at 280 nm (Figure 2). While hydrolyzable tannins were characterized by the high

concentration of monomeric phenols, grape-derived tannins contained high proportions of oligomeric and polymeric tannins that were based on flavonoids. Compared to hydrolyzable tannins, condensed tannins were characterized by a significant portion of absorbance due to the tannin fraction. HPLC analysis indicated a significant portion of total absorbance of grape skin tannin (S) and grape seed tannins (V, W) (Table 2, Figure 2). Chestnut tannin (C) and oak tannin (O) had 16% and 29% of total absorbance, respectively. Gallnut tannin (G) was constituted primarily of tannic acid, as reported elsewhere (Dumeau et al. 2004). Tannic acid had the same retention time of proanthocyanidins present in the seed and skin oenotannins (Figure 2).

**Characterization of grapes.** To establish the relationship between grape characteristics and the use of oenotannins in wine production, grapes from different cultivation areas and degrees of ripening were analyzed to determine their chemical profile. The characterization was performed in two years (2003 and 2004). The 2003 grapes harvested from two different areas (*a*, *b*) were similar in pH (3.54, 3.60) titratable acidity (5.18 g/L and 5.32 g/L), and sugar content (24.6 and 24.4 Brix) (Table 1). Phenolic richness ranged from 84 (grape group *a*) to 127 (group *b*) OD280 nm. Group *a* was lower in total potential anthocyanins (1197 mg/L) than group *b* (2737 mg/L). The cellular maturity index was lower for group *b* (37 EA%), indicating that this group had a higher ability of skin berries to release anthocyanins. Moreover, group *b* had higher skin tannin content (68).

For the 2004 harvest, the differences among the three harvest dates did not follow a systematic evolution (Table 1). Phenolic richness, total potential anthocyanins, extractable anthocyanins, seed maturity, and seed tannins decreased from the first to the second harvest date and then increased to the third date.

**Effect of time and type of oenotannin addition on 2003 wine color.** *Prefermentation addition.* Four oenotannins (two hydrolyzable: G, C; and two condensed: V, W) were added at 0.2 g/L to a Sangiovese must. Six months after the end of alcoholic fermentation, the wines were analyzed for color intensity (CI), hue (H), total phenol index (TPI), monomer anthocyanin

**Table 2** Chemical composition of six oenotannins, with manufacturer's recommended dose and pre- and postfermentation use.

Tannin	Recommended dose (g/hL)	Use	Total phenols <sup>a</sup>	Total proanthocyanidins <sup>b</sup>	mDP <sup>c</sup>	Tannins <sup>d</sup> (%)
Grape skin (S)	2–30	Postferment	617 c <sup>e</sup>	629 a	2.00a	55
Grape seed (V)	5–12	Preferment	596 b	776 b	4.07c	94
Grape seed (W)	3–30	Postferment	701 d	789 b	2.63b	56
Chestnut (C)	10–50	Preferment	527 a	nd		16
Oak (O)	2–20	Postferment	518 a	nd		29
Gallnut (G)	2–20	Pre-, postferment	873 e	nd		74
<b>LSD<sup>f</sup></b>			19.46***	65.92*	0.09*	

<sup>a</sup>mg equivalent gallic acid/g tannin.

<sup>b</sup>mg equivalent cyanidin/g tannin.

<sup>c</sup>Mean degree of polymerization.

<sup>d</sup>% of tannin content on total phenols.

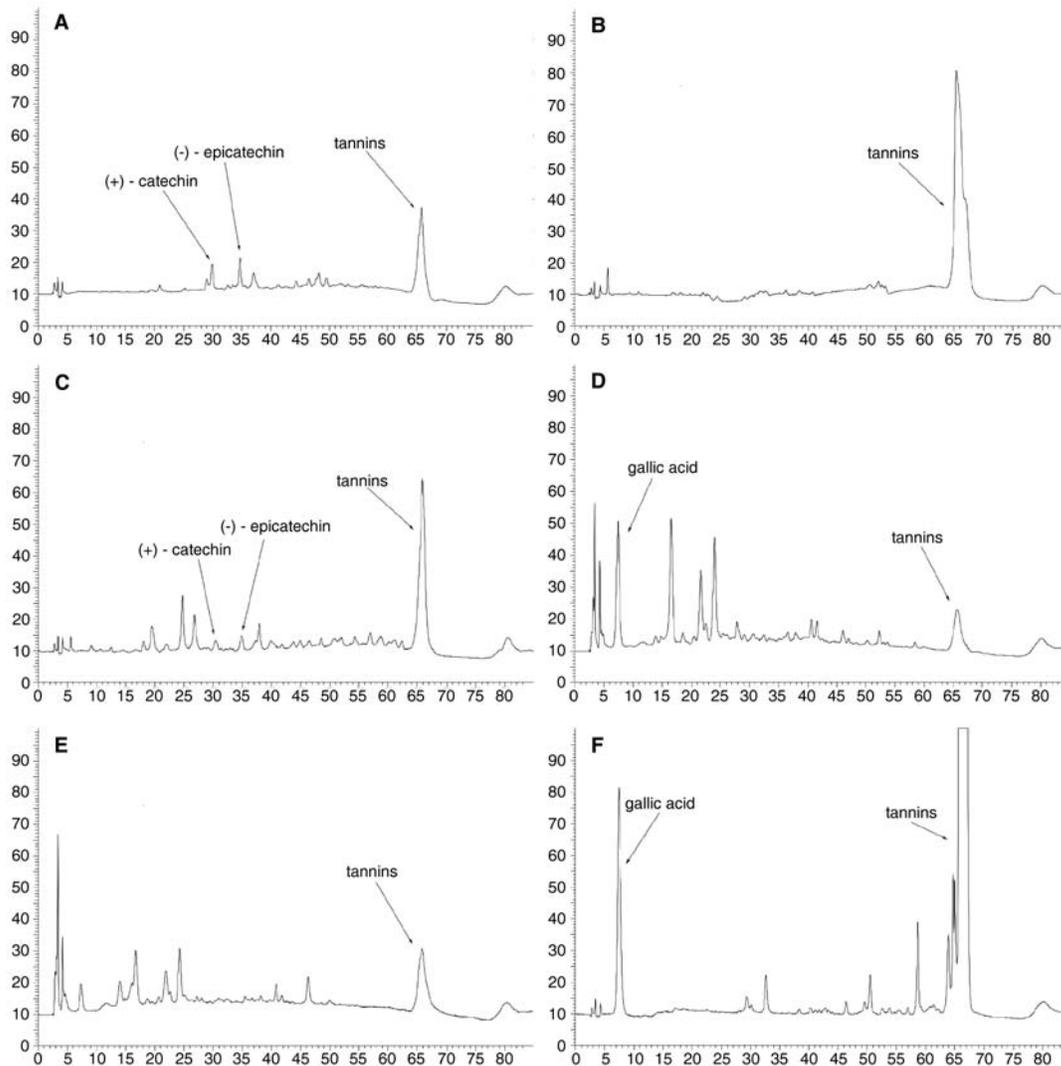
<sup>e</sup>Different letters within the same row indicate significant differences; nd: not detected.

<sup>f</sup>\* and \*\*\* indicate significance at  $p \leq 0.05$  and  $\leq 0.001$ , respectively.

content (M3MG), and colored polymeric pigments (CPP). All variables measured in wines obtained by using different oenotannins in prefermentation addition (TA) were significantly different among wines (Table 3). The influence of grape (g) on wine variables, except for TPI and CI, was significantly different. The replications of vinifications were significant only for M3MG, which might be dependent on a slight effect of different dissolved oxygen in must that could oxidize the free anthocyanins. The significant interactions were wine (TA) x grape (g) for all variables (except for TPI). These interactions were due to different grape composition, which influenced the effect of oenotannin addition.

Principal component analysis (PCA) was used to illustrate the relationships among the variables and wines (Figure 3). The first two principal components (PCs) accounted for 94% of the total variance. According to the test set validation, the two components were significant. The first PC contrasted CI, TPI, and CPP with M3MG and H. The second PC was defined by CI, TPI, M3MG, and H. The factor loadings (shown as

vectors) and the wines were plotted (Figure 3). Wines located on the left on the first PC (*bV*, *bC*) presented higher values for M3MG and H. In general, these wines were obtained by adding condensed oenotannin (from grape seed, V) and hydrolyzed tannin (from chestnut, C). In contrast, wines obtained from grape group *a* by adding hydrolyzed tannin (from gallnut G) *aG* and condensed oenotannin (from grape seed, W) *aW* were on the right and characterized by CI, TPI, and CPP. The wine distribution is related to grape characteristics and type of added oenotannin. In particular, the first PC contributed mainly to separate wines obtained from group *a* with addition of W and G oenotannins. The second PC separated mainly the wines obtained from groups *a* and *b*. The addition of oenotannins had only a minor effect on wines obtained from group *b*, which was richer in phenols than group *a*. The key finding was that tannins G and W had the most evident effect, especially on wines made from group *a*. These tannins stabilized the color and increased the CI. Wine *aW* had the highest CPP and CI.



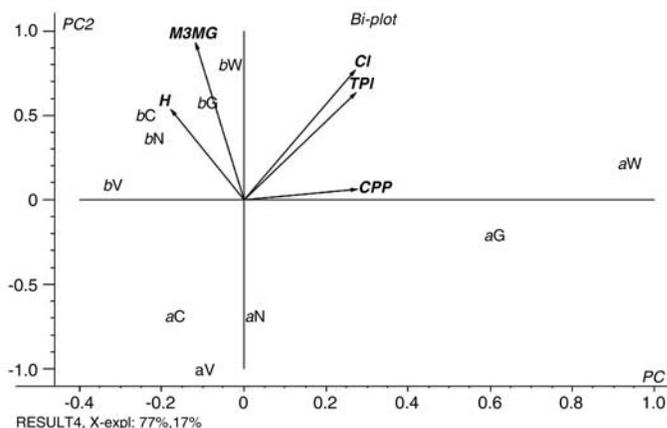
**Figure 2** HPLC chromatogram, showing absorbance at 280 nm, of the six oenotannin hydro-alcoholic solutions (1 g/L) highlighting the monomeric phenolic material [(+)-catechin and (-)-epicatechin] and tannins. **A:** grape skin tannin (S); **B:** grape seed tannin (V); **C:** grape seed tannin (W); **D:** chestnut tannin (C); **E:** oak tannin (O); **F:** gallnut tannin (G).

**Postfermentation addition.** The effect of three oenotannin additions on the color structure in postfermentation was assessed. Following the manufacturer information (Table 2), two condensed (S and W) and one hydrolyzed (O) oenotannins were used. Wines were obtained without adding oenotannins in the prefermentation phase and were analyzed 6 months after the end of malolactic fermentation. All measured variables except hue were significantly different among wines (TB) where different oenotannins were added (Table 4). The influence of grape (g) on wine variables was highly significant. The replications of vinifications were not significant. The only highly significant interactions were wine (TB) x grape (g) for all variables except hue. These interactions were due to the different composition of wines produced by grape groups *a* and *b*.

According to PCA, the first two PCs accounted for 95% of the total variance (Figure 4). The first PC grouped all variables, except CPP, to the right of the axis and the second PC was defined by CPP. The first PC separated the wines obtained from groups *a* and *b* and the second PC separated the wines with added oenotannins. There was only a weak influence among wines obtained with (NW, NO, NS) and without (NN) oenotannin additions.

**Pre- and postfermentation addition.** Oenotannin addition had an important influence on wine color both pre- and postfermentation, and the characteristics of the grapes influenced the effect of different tannins (Table 5). The interaction between TA and grapes (g) was significant for all variables; however, the interaction between TB and grapes (g) was significant for CI and CPP only. The combination of different oenotannins added at different stages of winemaking (TA x TB) was significant for CI, H, and CPP only.

PCA was used to illustrate the relationships among the variables and wines (Figure 5). The first two significant PCs accounted for 93% of the total variance. The first PC contrasted CI, TPI, and CPP with M3MG and H, while the second PC was defined by all variables that were located in the positive plane of the y axis. Wines located in the positive plane of the second PC presented higher values for all variables. In general, these wines were obtained from grape group *b*. In contrast, wines obtained from grape group *a* were located in the negative plane. The first PC separated the wines obtained



**Figure 3** Effect of oenotannin addition prefermentation. Relationship between the first two PC scores of the phenolic parameters (vectors) of wine samples 6 months after the end of malolactic fermentation (mean values) (*a*, *b* wines obtained from 2003 harvest grapes; N, no tannin addition (control); C, chestnut tannin; G, gallnut tannin; V and W, grape seed tannin; TPI, total phenol index; CI, color intensity; H, hue; M3MG, total free anthocyanins expressed as malvidin-3-monoglucoside; CPP, colored polymeric pigments expressed as malvidin-3-monoglucoside).

**Table 3** F values and interaction for phenolic compounds of wines made from grape groups *a* and *b* obtained from the 2003 harvest with prefermentation addition of tannin after 6 months of aging.

Variable <sup>a</sup>	TA	g	r	TA x g	TA x r	g x r
TPI	7.10 <sup>nb</sup>	0.00 ns	3.11 ns	1.04 ns	0.46 ns	0.03 ns
CI	64.00 <sup>***</sup>	0.28 ns	0.74 ns	43.43 <sup>**</sup>	12.03 <sup>*</sup>	4.26 ns
H	1116.00 <sup>***</sup>	4761.00 <sup>***</sup>	1.00 ns	826.00 <sup>***</sup>	1.00 ns	1.00 ns
M3MG	64.86 <sup>***</sup>	2418.29 <sup>***</sup>	15.77 <sup>*</sup>	39.38 <sup>**</sup>	0.65 ns	0.02 ns
CPP	616.31 <sup>***</sup>	647.43 <sup>***</sup>	0.94 ns	310.16 <sup>***</sup>	3.25 ns	5.34 ns

<sup>a</sup>Abbreviations: TA, prefermentation tannin; g, grape; r, replicates; TPI, total phenol index; CI, color intensity; H, hue; M3MG, total amount of free anthocyanins expressed as malvidin-3-monoglucoside; CPP, colored polymeric pigments expressed as malvidin-3-monoglucoside. <sup>b</sup>\*, \*\*, \*\*\*, and ns indicate significance at  $p \leq 0.05$ ,  $\leq 0.005$ ,  $\leq 0.001$ , and not significant, respectively.

**Table 4** F values and interaction for phenolic compounds of wines made from grape groups *a* and *b* obtained from the 2003 harvest with postfermentation addition of tannin after 6 months of aging.

Variable <sup>a</sup>	TB	g	r	TB x g	TB x r	g x r
TPI	97.00 <sup>**b</sup>	361.00 <sup>***</sup>	1.00 ns	35.67 <sup>**</sup>	1.00 ns	1.00 ns
CI	150.33 <sup>***</sup>	3025.00 <sup>***</sup>	1.00 ns	33.00 <sup>**</sup>	1.00 ns	1.00 ns
H	0.41 ns	361.00 <sup>***</sup>	0.11 ns	0.41 ns	0.41 ns	0.11 ns
M3MG	24.86 <sup>*</sup>	115.54 <sup>**</sup>	0.05 ns	43.44 <sup>*</sup>	2.57 ns	5.01 ns
CPP	126.05 <sup>**</sup>	23076.49 <sup>***</sup>	1.34 ns	124.52 <sup>**</sup>	1.07 ns	1.21 ns

<sup>a</sup>Abbreviations: B, postfermentation tannin; g, grape; r, replicates; TPI, total phenol index; CI, color intensity; H, hue; M3MG, total amount of free anthocyanins expressed as malvidin-3-monoglucoside; CPP, colored polymeric pigments expressed as malvidin-3-monoglucoside. <sup>b</sup>\*, \*\*, \*\*\*, and ns indicate significance at  $p \leq 0.05$ ,  $\leq 0.005$ ,  $\leq 0.001$ , and not significant, respectively.

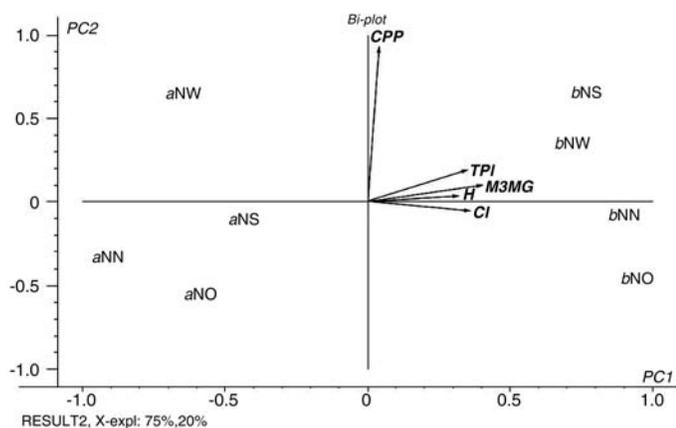
from group *a* in relation to different oenotannins added during prefermentation. A less important influence was observed for wines obtained from group *b*.

It is important to emphasize the similarity between Figures 3 and 5, which show that the wines obtained from grape groups *a* and *b* were clearly separated in both graphical representations. Another evidence of similarity was the influence of oenotannin G and W added during prefermentation in determining the difference among the wines even when oenotannins were added during postfermentation. The moderate effect on color characteristics can be explained considering that a large portion of colored polymeric pigments are formed from anthocyanins and tannins during alcoholic fermentation (Eglinton et al. 2004). The wine distribution indicates that preferment addition is the most influential in determining final color characteristics.

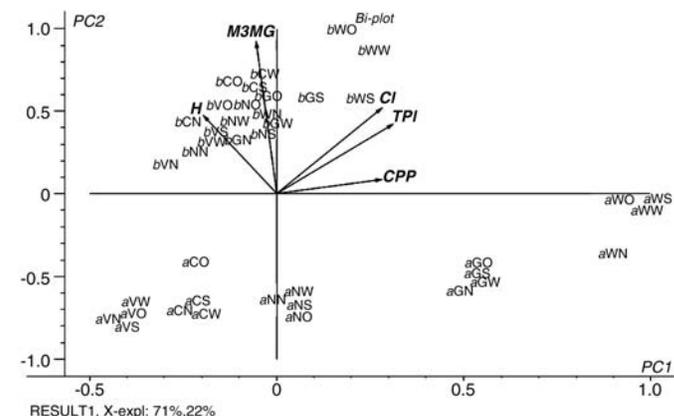
**Effect of grape ripening and oenotannin addition on 2004 wine color.** *Prefermentation addition.* Oenotannins were added to the must of grape group *c* harvested at three different ripeness levels. Two oenotannins were tested, one condensed (W) and one hydrolyzed (G), which showed a sig-

nificant effect on the color of wines during the 2003 harvest. All measured variables were significantly different among the wines prepared with different oenotannins (TA) (Table 6). The influence of grape ripening (g) on wine variables was highly significant. The replications of vinifications were significant only for M3MG. The only highly significant interaction was wine (TA) x grape (g) for CI, CPP, and M3MG. This interaction was due to different content of extractable anthocyanins (Table 1).

According to PCA, the wine configuration for the first two significant PCs accounted for 92% of the variance (Figure 6). The only pattern apparent in the distribution of the wines was the separation of the wines along the first PC on the basis of ripening degree. The first PC contrasted CPP, CI, and TPI with M3MG and H. The second PC was defined by M3MG and H. Wines located on the right of the first PC—*c*3N, *c*3G, and *c*3W—presented higher values of CPP, CI, and TPI and were obtained from the third level of grape ripening. This distribution indicated that oenotannin addition had a slight effect on wines obtained from the first and second levels of grape ripening, which were less rich in phenols than grapes



**Figure 4** Effect of oenotannin addition postfermentation. Relationship between the first two PC scores of the phenolic parameters (vectors) of wine samples 6 months after the end of malolactic fermentation (mean values) (*a, b*, wines obtained from 2003 harvest grapes; NN, no tannin addition (control); NW, grape seed tannin; NS, grape skin tannin; NO, oak tannin; TPI, total phenol index; CI, color intensity; H, hue; M3MG, total free anthocyanins expressed as malvidin-3-monoglucoside; CPP, colored polymeric pigments expressed as malvidin-3-monoglucoside).



**Figure 5** Effect of oenotannin addition prefermentation (N, control; V and W, grape seed tannin; C, chestnut tannin; G, gallnut tannin) and postfermentation (N, control; S, grape skin tannin; W, grape seed tannin; O, oak tannin). Relationship between the first two PC scores of the phenolic parameters (vectors) of wine samples 6 months after the end of malolactic fermentation (mean values) (*a, b*, wines obtained from 2003 harvest grapes; TPI, total phenol index; CI, color intensity; H, hue; M3MG, total free anthocyanins expressed as malvidin-3-monoglucoside; CPP, colored polymeric pigments expressed as malvidin-3-monoglucoside).

**Table 5** F values and interaction for phenolic compounds of wines made from grape groups *a* and *b* obtained from the 2003 harvest with prefermentation and postfermentation addition of tannin after 6 months of aging.

Variable <sup>a</sup>	TA	TB	g	r	TA x g	TB x g	TA x TB	g x r	TA x r	TB x r
TPI	249.09*** <sup>b</sup>	28.01***	10.68**	5.76*	40.65***	0.96 ns	0.58 ns	0.06 ns	0.85 ns	5.76 ns
CI	323.36***	9.34***	25.99***	0.12 ns	147.45***	4.29**	3.28**	1.43 ns	2.97*	0.20 ns
H	1196.44***	25.45***	4609.86***	0.00 ns	1049.98***	1.13 ns	4.17**	0.00 ns	0.33 ns	0.17 ns
M3MG	66.66***	5.38**	2311.17***	1.09 ns	12.98***	1.27 ns	1.01 ns	0.00 ns	0.06 ns	0.93 ns
CPP	337.23***	14.49***	109.71***	0.10 ns	134.87***	7.29***	2.60*	0.27 ns	0.27 ns	0.07 ns

<sup>a</sup>Abbreviations: TA, prefermentation tannin; TB, postfermentation tannin; g, grape; r, replicates; TPI, total phenol index; CI, color intensity; H, hue; M3MG, total amount of free anthocyanins expressed as malvidin-3-monoglucoside; CPP, colored polymeric pigments expressed as malvidin-3-monoglucoside.

<sup>b</sup>\*, \*\*, \*\*\*, and ns indicate significance at  $p \leq 0.05$ ,  $\leq 0.005$ ,  $\leq 0.001$ , and not significant, respectively.

**Table 6** F values and interaction for phenolic compounds of wines made from grape group *c* (1, 2, 3 levels of ripeness) obtained from the 2004 harvest with the prefermentation addition of tannin after 6 months of aging.

Variable <sup>a</sup>	TA	g	r	TA x g	TA x r	g x r
TPI	205.30*** <sup>b</sup>	1420.91***	0.02 ns	2.11 ns	5.56*	33.20**
CI	25.13***	405.80***	0.53 ns	3.70***	1.63 ns	15.98*
H	3.42*	9.66***	1.10 ns	1.96 ns	4.40*	1.10 ns
CPP	60.02***	1118.43***	0.02 ns	17.69***	3.91*	35.39*
M3MG	9.16**	76.86***	124.00***	23.09***	16.93*	57.84*

<sup>a</sup>Abbreviations: TA, prefermentation tannin; g, grape; r, replicates; TPI, total phenol index; CI, color intensity; H, hue; M3MG, total amount of free anthocyanins expressed as malvidin-3-monoglucoside; CPP, colored polymeric pigments expressed as malvidin-3-monoglucoside. b\*, \*\*, \*\*\*, and ns indicate significance at  $p \leq 0.05$ ,  $\leq 0.005$ ,  $\leq 0.001$ , and not significant, respectively.

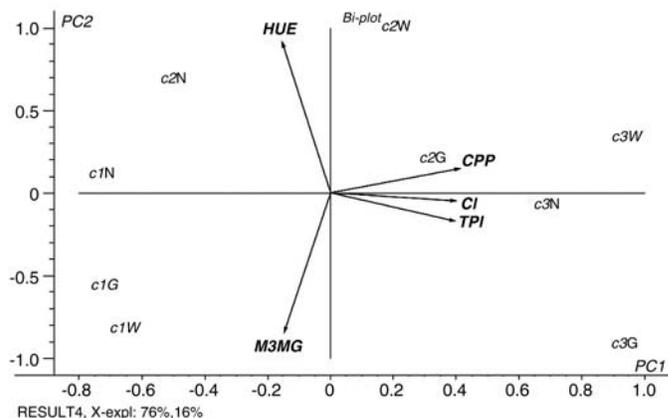
from the third level. The most important finding was that tannins G and W had the most evident effect. Wine *c3W* and *c3G* had the highest CPP and CI (Figure 6).

*Relationship between grape maturity indices and wine color parameters.* The relationship between some indices measured in the 2004 grapes and wine color attributes was examined using the soft modeling technique of partial least square (PLS) regression analysis. This procedure attempts to account for any common variation between two blocks of data. Grape maturity indices were used as explanatory variables while wine phenolic parameters were simulated as dependent variables (Table 7). The single latent variable explained ~71% of the wine color indice (Y) variance. The wine color indices were related positively to total potential anthocyanins (ApH1.0), phenolic richness (OD280 nm), seed maturity index (%), seed tannins (DT<sub>pep</sub>), and skin tannins (DT<sub>pell</sub>) and negatively to cellular maturity index (EA%).

## Discussion

**Characterization of oenotannins.** The mean degree of polymerization (mDP) for grape seed tannins was 2.63 and 4.07 for W and V oenotannins, respectively, values that agree with a study which found that commercial seed products may have a range of 1.42 to 6.81 mDP (Nadège and Bertrand 2005). Grape skin tannin (S) had 2.0 mDP, a value in accordance with a study that found commercial grape skin oenotannins have 1.8 mDP (Vivas et al. 2004). A low mDP value for commercial grape seed and skin tannins depends on the extraction process. Most oenotannins derived from grape material are extracted using water rather than an organic solvent. Condensed tannins account for 45% of the total phenol extract when an organic solvent is used compared to ~13% when an aqueous solvent is used. Aqueous solvents extract only low molecular weight proanthocyanidins, which would explain the low mDP of skin-derived oenotannins despite skin tannins containing high molecular weight proanthocyanidins (Nadège and Bertrand 2005).

By adding the same amount of commercial product to wine, it is apparent that we added different amounts of tannin material (Table 2). The variability among commercial oenotannins could be high, both in terms of the proportion of phenolic to nonphenolic material and the proportion of tannic to nontannic phenolics, as reported elsewhere (Sarneckis et al. 2006).



**Figure 6** Influence of grape maturity and prefermentation tannin addition. Relationship between the first two PC scores of the phenolic parameters (vectors) of wine samples 6 months after the end of malolactic fermentation (mean values) (*c* wines obtained from 2004 harvest grapes; 1, 2, 3, levels of ripeness; N, no tannin addition (control); G, gallnut tannin; W, grape seed tannin; TPI, total phenol index; CI, color intensity; H, hue; M3MG, total free anthocyanins expressed as malvidin-3-monoglucoside; CPP, colored polymeric pigments expressed as malvidin-3-monoglucoside).

**Characterization of grapes.** The values for measured parameters were considered suitable for the current target enological model of Sangiovese wines (Bucelli et al. 2010). The differences in composition between groups *a* and *b* (2003 harvest) were determined by the characteristics of the soil. The growing area of group *a* was characterized by clay and rocky soil, while the growing area of group *b* had deposits of alluvial material with a deep rooting potential. The evolution of sugar and acidity contents during maturation was in accordance with other research on Sangiovese (Storchi et al. 2005, Poni et al. 2008). Regarding the phenol fractions of 2004 grapes, the differences among the three harvesting dates did not follow a systematic evolution (Table 1), which could be linked to the nighttime temperature and sunlight days during ripening. In particular, from 20 Sept to 5 Oct the nighttime temperatures were lower than average (<15°C instead of 18°C) with 12 cloudy days. The effect of these environmental factors on grape composition has been reported (Cohen and Kennedy 2010). The composition of grapes harvested on 20 Sept and 5 Oct was considered slightly suitable for Sangiovese wines, whereas the composition of grapes harvested on 19 Oct was considered more suitable (Bucelli et al. 2010).

**Table 7** PLS analysis: relationship between grape maturity indices (x data) and wine color indices (y data) loading plot.

Grape maturity indices (x)	Loading
Total potential anthocyanins (ApH1.0, mg/L M3MG)	0.402
Cellular maturity index (EA%)	-0.410
Phenolic richness (DO280 nm)	0.484
Seed maturity %	0.285
Skin tannins % (DTpell)	0.419
Seed tannins % (DTpep)	0.432
Wine color indices (y) <sup>a</sup>	
TPI	0.350
CI	0.078
H	0.455
CPP	0.469
M3MG	0.482
Explained variance	%
x versus y	71

<sup>a</sup>TPI, total phenol index; CI, color intensity; H, hue; M3MG, total amount of free anthocyanins expressed as malvidin-3-monoglucoside; CPP, colored polymeric pigments expressed as malvidin-3-monoglucoside.

**Effect of different time and type of oenotannin addition on 2003 wine color.** *Prefermentation addition.* The wines obtained with prefermentation condensed tannin addition had higher TPI and CPP than the control wines and the wines obtained with the addition of gallotannins during prefermentation, results that agreed with others (Bautista-Ortín et al. 2005). One study did not identify differences in wines during fermentation and after malolactic fermentation (Parker et al. 2007), while another found differences during fermentation but not after malolactic fermentation (Bautista-Ortín et al. 2005). Tannins V and C had no influence on the color and the wines were similar to control N wine. This effect might be explained by the low reactivity of these oenotannins determined by a low concentration of tannin (hydrolyzed oenotannin C) and low reactivity of proanthocyanidins present in condensed tannin V (Table 2). In agreement with Kahn and Bertrand (2005), the reactivity of condensed tannin can be inversely related to the mean degree of polymerization (mDP).

*Postfermentation addition.* The addition of oenotannins during postfermentation had a slight effect on wines obtained from grapes *a* and *b* (Figure 4). The addition of different oenotannins showed no selective effect on wine composition, and the wines with oenotannin addition were located closely in the biplot. The slight effect of addition of oenotannins during postfermentation has also been reported elsewhere (Parker et al. 2007).

*Pre- and postfermentation addition.* The combination of pre- and postfermentation tannin addition made it possible to understand the influence of the different grape characteristics and the effectiveness of oenotannin addition. These results might give insight into the use of oenotannins, as the effect was more important in pre- rather than postfermentation. The moderate effect of oenotannin additional on color characteristics can be explained, according to Eglinton et al. 2004, by the lower kinetics of aggregation between anthocyanins

and tannins, to form colored polymeric pigments, during fermentation.

Only two oenotannins (G and W) stabilized and increased wine color. These oenotannins were characterized by higher total phenols and higher proanthocyanidins (only for condensed oenotannin W) (Table 2, Figure 2C, 2F). In particular, the low degree of polymerization of oenotannin W (2.63 mDP) could led to higher reactivity with anthocyanins, compared with seed condensed tannin (V). A high correlation between the degree of polymerization of oenotannins and active tannin fraction was reported by Kahn and Bertrand 2005.

**Effect of grape ripening and oenotannin addition on 2004 wine color.** *Prefermentation addition.* Oenotannins were added to the must of grapes harvested at three different ripeness levels. Grape ripeness is the major factor affecting anthocyanin accumulation in grape skin. The extractability of anthocyanins typically increases throughout grape ripening (sugar accumulation), while the extractability index decreases as maturation progresses (Saint-Criq et al. 1998, Glories 1999). Our findings are in contrast, as the extractability index increased with increasing grape maturity (sugar content). However, previous studies reported that the sugar content increased during ripening with no significant changes in the extractability index (González-Neves et al. 2002).

As with the 2003 harvest, tannins G and W also showed efficacy for the 2004 harvest during prefermentation. In a study with Shiraz, the formation of approximately half of the pigmented polymer concentration was achieved during the fermentation period alone (Parker et al. 2007); the authors highlighted the importance of this relatively short period in maximizing pigment polymer concentration, which is important to long-term red wine color.

*Grape maturity indices and wine color parameters.* Recent studies have investigated the relationship between grape phenolics and wine color (Jensen et al. 2008, González-Neves et al. 2010). The results have demonstrated that it is possible to predict the color characteristics of wine from grape measurements, and thus they provide an important starting point for further identification and prediction of wine quality parameters from grape measurements. Here we observed that the polyphenolic richness of the grape (grape maturity index) contained information that makes it possible to predict the color parameters of wine (Table 7). In our current research we are evaluating the two distinct phases in a data-driven predictive model: training (or calibration) and prediction. In the calibration phase, the model for predicting wine variables from grape variables is determined based on empirical data and prior knowledge. In the prediction phase, the model with “known” parameters is applied to data from grape variables in new samples in order to predict the “unknown” value of the wine parameters. The grape maturity indices seem to be reliable in tracing the positive modification in wine when specific treatments were considered, such as oenotannin addition.

## Conclusion

This study has identified the potential to manage the addition of oenotannins depending on the phenolic composition of

the grapes. Wines obtained from grapes with high phenolic concentration were less influenced by tannin addition during prefermentation. The timing of oenotannin addition had a different effect on color stability: addition during prefermentation had a more significant influence on color structure than did addition during postfermentation. Seed grape condensed tannin (V) and gallnut tannin (G) had a greater influence than the other oenotannins (S, W, C, O) on color stabilization.

For Sangiovese, the polyphenolic richness of the grape (grape maturity index) contains information that could make it possible to predict the color parameters of wine. In a future study, we aim to develop a model for the tailored use of tannins to provide wines with a controlled stability, with subsequent improvement in the quality of the available oenotannins. More generally, the knowledge of the polyphenolic richness of the grapes and their extractability allows better control of wine-making technologies and operational conditions. We hope that further studies will be able to elucidate the specific phenolic compounds of grapes, which may in turn explain the relationship among grape characteristics, tannin addition, and wine sensory properties.

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