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# Profiling of Hydroxycinnamoyl Tartrates and Acylated Anthocyanins in the Skin of 34 *Vitis vinifera* Genotypes

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**ABSTRACT:** The diversity of berry skin flavonoids in grape genotypes has been previously widely investigated with regard to major compounds (nonacylated anthocyanins and flavonols), but much less with regard to acylated anthocyanins and hydroxycinnamoyl tartrates (HCTs). In this study, the composition of the phenolic fraction of the berry skin (free and acylated anthocyanins, flavonols, and HCTs) was assessed on 34 grapevine genotypes grown in a collection vineyard in northwestern Italy. The phenolic fraction was profiled on berries collected in the same vineyard, at the same ripening level across two successive vintages. The anthocyanin, HCT, and flavonol profiles were specific of each genotype, and the first two were relatively little affected by the vintage. A wide diversity in the polyphenolic fraction was shown among cultivars. Besides expected discriminatory effects of free anthocyanins and flavonol profiles, principal component analyses allowed a good discrimination of cultivars on the basis of coumaroylated anthocyanins and of the HCT profile. Anthocyanins were mostly acylated by aromatic acids, and acylation was independent from the anthocyanin substrate. HCTs were present mostly as coumaroyl and caffeoyl derivatives, and no correlation was observed between the same acylation patterns of tartrate and of anthocyanins. The results of this study are discussed in the light of new hypotheses on still unknown biosynthetic steps of phenolic substances and of the potential use of these substances in discrimination and identification of different grape cultivars in wines.

**KEYWORDS:** *polyphenols, HPLC-DAD, principal component analysis, chemometrics*

## INTRODUCTION

*Vitis vinifera* berries are rich in flavonoids such as anthocyanidins (in colored grapes), flavonols, flavan-3-ols, and proanthocyanidins and in nonflavonoid phenols such as hydroxycinnamoyl tartrates (HCTs). Flavonol and HCT concentrations are second to proanthocyanidins and anthocyanidins in berry skins, whereas in berry pulps, apart from anthocyanin-containing red-fleshed grapes, HCTs are considered to be the most abundant phenolics,[1,2] followed by monomeric and oligomeric flavan-3-ols.[3]

Anthocyanins are present in the grapevine berry skin as 3-monoglucosides of five differently hydroxylated and *O*-methylated anthocyanidins, but the diversity of their chemical forms is greatly increased by acylation in the C6-position of the glucose moiety. Aliphatic (acetyl) and aromatic (coumaroyl and caffeoyl) acids are the substrates of the enzymes catalyzing anthocyanin acylation. Anthocyanins are the base of red wine color and perform complex interactions with other phenolic substances under oxidative conditions during winemaking and wine aging.[4,5] The biosynthesis of anthocyanidins and their glycosylation pathways are relatively well-known,[6,7] and a few genes that decorate anthocyanins with hydroxyl and methyl groups have been described,[8,9] whereas no genes or enzymes catalyzing the acylation step have been discovered up to now.

Flavonols are predominantly localized in the berry skins of both white and colored grapes. From a biological point of view, their role seems to be linked to UV screening[10] and, technologically, they are involved in the color stabilization of red wines, through copigmentation phenomena,[11] and in the sensory perception of bitterness, at least in model tea solution.[12] Flavonols are found in grape berry skins as 3-glycosides (glucosides, glucuronides, and galactosides); the main flavonols reported in grape berries are the dihydroxylated quercetin and the trihydroxylated myricetin, but other compounds such as the monohydroxylated kaempferol and the methylated isorhamnetin, laricitrin, and syringetin have also been identified.[13,14] Two recent comprehensive works by Castillo-Muñoz and coworkers[15,16] have established the complete series of 3-glucosides, glucuronides, and galactosides of six flavonol aglycons (kaempferol, quercetin, isorhamnetin, myricetin, laricitrin, and syringetin) in red varieties and of three aglycons (quercetin, kaempferol, and isorhamnetin) in white varieties.

The biosynthesis of flavonols takes place as a side branch of anthocyanin biosynthesis, via reduction of dihydroflavonols by the action of flavonol synthase.[17] The diversity of flavonols is mostly due to hydroxylation reactions at the B ring, which take place at the dihydroflavonol level, and to a lesser extent to *O*-methylation. In grape, hydroxylases and a methyltransferase that could be responsible for such processes have been isolated.[8,9] Flavonol glycosylation could be explained by the side activity of the same glycosyltransferase acting on anthocyanidins,[18] but no genes responsible for glucuronylation have been discovered up to now.

Hydroxycinnamoyl tartrates (HCTs) are the most abundant group of nonflavonoid phenols in grapes and wines. The

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predominant HCTs in *V. vinifera* grape berry pulps and skins are caffeoyltartaric (caftaric) acid, *p*-coumaroyltartaric (coutaric) acid, and feruloyltartaric (fertaric) acid, the *trans* isomers of which are much more abundant than the *cis* forms.[2] Concentrations of HCTs in juices of different *V. vinifera* cultivars are highly variable, ranging from a few milligrams per liter to several hundreds of milligrams per liter.[1] HCTs, known to be involved in the browning reactions of must and wine,[19] are precursors of volatile phenols and possess antimicrobial and antioxidant properties.[20] In wines, phenolic acids, which can originate from hydrolysis of HCTs, contribute to sensory perception by enhancing astringency;[21] besides, they have been shown to be of great significance in the taxonomy of young single-variety wines.[22] In addition, they take part in the formation of derived pigments with anthocyanins and contribute to color stabilization in aging wines.[23] The biosynthetic pathway of HCTs in grapevine is not known, although the biosynthesis of the related caffeoylquinic (chlorogenic) acid, which is not normally recorded in grapevine, has been clarified in tobacco.[24,25]

Diversity within the grape species is expressed in thousands of vegetatively propagated genotypes differing in the concentration of the various classes of phenolics and in their phenolic profiles (i.e., the relative concentration of individual phenolic compounds). The wide diversity in wine grape flavonoid composition is of major technological importance, each cultivar requiring dedicated enological adaptation of the winemaking techniques. This diversity can also be exploited for chemotaxonomic purposes, with the aim to identify compounds that can help to single out specific genotypes, to be used both for basic studies and for the assessment of the varietal composition of wines, considering the relative stability of some of these molecules during vinification. Finally, the study of metabolic profiles is also of biological interest, as it yields indirect information on the mechanisms underlying the biosynthesis of the different compounds. For these reasons, the study of phenolic profiles in different grape genotypes has been extensively followed focusing, in particular, on nonacylated anthocyanins and flavonols, whereas very few studies on a wide genotype range of *V. vinifera* have been performed with regard to HCTs.

In this study we profiled the anthocyanin (free and acylated), flavonol, and HCT fractions of berry skins in a set of 34 not yet or poorly characterized *V. vinifera* cultivars over a period of two years. Among the studied genotypes, 7 had non-colored berries, 2 had pale-rose berries, 22 had colored berries, and 3 were red-fleshed cultivars (accumulating anthocyanins both in skin and in pulp). We focused our attention in particular on the HCT fraction and on the patterns of anthocyanin acylation.

## MATERIALS AND METHODS

**Plant Material.** The berries of 34 *V. vinifera* cultivars were sampled in two consecutive years (2006 and 2007) in the collection vineyard located at Grinzane Cavour (Cuneo province, Italy; http://www.ivv.cnr.it/new/grinzane/index.htm). In the experimental vineyard the 2006 vegetative season (April−September) was cooler than the corresponding period in 2007 (the summations of daily average temperatures >10 °C were 1893 and 2131 °C, respectively), with differences concentrated in the period before véraison (1199 °C from April to July in 2006 against 1503 °C in the same period in 2007). The 2006 vegetative season witnessed also a lower cumulated solar irradiation in the 400−700 nm range (1576 MJ $m^{-2}$) than the corresponding 2007 period (1707 MJ $m^{-2}$).

The collection vineyard was planted in 1992 with the aim of maintaining minor local cultivars from the Italian regions of Piedmont, Liguria, and Aosta Valley, together with other Italian and international reference cultivars. The 34 genotypes chosen for the analyses included 24 minor, locally grown, cultivars, for which the berry phenol composition had not been analyzed in detail yet; 3 major Italian cultivars (Barbera, Dolcetto, and Nebbiolo), and 7 international cultivars (Cabernet sauvignon, Chardonnay, Chasselas blanc, Moscato bianco = white muscat, Moscato d'Amburgo = Muscat of Hambourg, Alicante Bouschet, Pinot noir) (Table 1). Vines were trained to a vertical trellis system and Guyot pruned. Canopies were routinely managed during spring and summer according to the standard cultural practices of the cultivation area. In addition, crop load was controlled and standardized with cluster removal in the prevéraison period. For each variety and in both years, berries were collected when they had reached a total soluble solids content (SSC) of 20 ± 1 °Brix.

In the vineyard every cultivar was present as duplicate plots of 10−20 vines. After a preliminary measurement of soluble solids performed directly in the vineyard on 10 berries per plot for each cultivar, if the SSC was 20 ± 1 °Brix, about 25 berries from each plot were collected for each cultivar, from the upper, middle, and bottom parts of the clusters and the shaded and exposed sides of the row, and pooled together. The SSC of 20 berries was measured again in the laboratory, and only if both measurements (the one in the vineyard and the one in the laboratory) ranged from 19 to 21 °Brix were the remaining collected berries divided into three subgroups of 10 berries each and used as triplicates for anthocyanin, flavonol, and HCT measurements. This sampling protocol brought to scalar harvests, as detailed in Table 1. The 10-berry samples were processed as described in ref 26. Briefly, skins were manually separated from seeds and pulps and extracted in a pH 3.2 ethanol buffer containing 2 g/L of $Na_2S_2O_5$ at 30 °C for 72 h.

**Analysis of Anthocyanins.** Anthocyanins were separated by applying the supernatant diluted 1:1 with 0.05 M sulfuric acid onto a 1 g Sep-Pak $C_{18}$ cartridge (Waters Corp., Milford, MA, USA) and were eluted with methanol. The methanolic extract was evaporated to dryness using an R-200 rotating evaporator (Büchi, Flawil, Switzerland) under reduced pressure at 35 °C and resuspended in solvent B used for HPLC analysis. All extracts were filtered through a 0.20 μm PTFE filter (Millipore Corp.).

Total anthocyanins were assessed by using a UV-1601PC spectrophotometer (Shimazdu Scientific Instruments Inc., Columbia, MD, USA) and expressed as malvidin 3-*O*-glucoside equivalents. The profile of glucosylated anthocyanin was determined by HPLC-DAD analyses, using a P100 instrument equipped with a Spectra Focus diode array detector operating at 520 nm, an AS3000 autosampler, and a 20 μL Rheodyne sample loop (Spectra Physics Analytical Inc., San Jose, CA, USA). Chromatographic separation was carried out using a LiChroCart analytical column (25 cm × 0.4 cm i.d.) purchased from Merck (Darmstadt, Germany), packed with LiChrosphere 100 RP-18 (5 μm) particles supplied by Alltech (Deerfield, IL, USA). Chromatographic conditions were those used in a previous work;[27] briefly, the solvents used were A = 10% formic acid in water and B = 10% formic acid and 50% methanol in water. Solvent flow rate was 1 mL/min. The following solvent A proportions were used: from 72 to 55%, 15 min; to 30%, 20 min; to 10%, 10 min; to 1%, 5 min; to 72%, 3 min. Data treatment was carried out using the ChromQuest chromatography data system (ThermoQuest, Inc., San Jose, CA, USA). Nonacylated anthocyanins were identified by comparison with pure standards purchased from Extrasynthèse (Genay, France), when available. The remaining anthocyanins were identified by matching the DAD spectrum and retention time of each chromatographic peak with available data in the literature.[28] The percentages of individual anthocyanins were determined by comparing the area of the individual peak with the total peak area.

**Analysis of Flavonols and HCTs.** The 10-berry skin extract was diluted 1.1-fold with 1 M phosphoric acid. Extracts were filtered through 0.2 μm GHP membrane filters (Pall Corp., New York, NY, USA). Flavonols and HCTs were detected by a HPLC−diode array detector (DAD) system (Perkin-Elmer series 200-L pump) equipped with a LiChrosphere 100 RP-18 5 mm (25 × 0.4 cm i.d.) column with a LiChrocart C18 guard column (Merck, Darmstadt, Germany). As previously reported[26] solvent A (phosphoric acid $10^{-3}$ M) and solvent

**Table 1. Grape Genotypes Profiled in This Study, Their Geographic Distribution and Main Characteristics, and Dates of Harvest in the Two Years of Study[a]**

| | distribution[b] | notes | harvest date | |
|---|---|---|---|---|
| | | | 2006 | 2007 |
| Alicante Bouschet (ab) | I | well-known red flesh grape variety bred by H. Bouschet in 1865 crossing Grenache (Alicante) and Petit Bouschet | 28/09 | 12/09 |
| Arneis (arn) | L | reputed specialty of central Piedmont giving flavored, character full wines | 12/09 | 28/08 |
| Avanà (av) | L* | ancient alpine variety called Hibou in France | 12/09 | 28/08 |
| Barbarossa (from Piedmont) (buv) | L | ancient cultivar, threatened with extinction, giving beautiful coral-colored grapes for table use | 19/09 | 05/09 |
| Barbera (brb) | I | major wine grape from Piedmont, grown also in other Italian regions as well as overseas | 19/09 | 28/08 |
| Becuét (bec) | L* | old variety from the western Alps, also known as Persan in France, giving acidic, deep-colored, and well-structured wines | 12/09 | 28/08 |
| Brachetto (brA) | L | aromatic grape from southeastern Piedmont, used for popular sweet fizzy or sparkling wines | 05/09 | 21/08 |
| Brachetto Roero (brR) | L | aromatic grape from the area of Roero (central Piedmont), traditionally used for table consumption and for producing dry wines | 05/09 | 21/08 |
| Cabernet Sauvignon (cs) | I | | 19/09 | 05/09 |
| Chardonnay (ch) | I | | 05/09 | 21/08 |
| Chasselas blanc (chsb) | I | | 05/09 | 05/09 |
| Cortese (co) | L | major white variety in Piedmont | 12/09 | 28/08 |
| Croatina (cro) | It | quite important wine cultivar, mainly grown in Piedmont, Lombardy, and Emilia | 12/09 | 12/09 |
| Dolcetto (dlc) | L | one of the most planted reds in Piedmont, giving varietal-colored wines of medium body | 12/09 | 05/09 |
| Freisa (fre) | L | local variety from Piedmont, grown to a minor extent all over the region | 12/09 | 28/08 |
| Gambarossa (gro) | L | from a restricted area near Asti, giving spicy, medium-bodied wines | 19/09 | 05/09 |
| Grignolino (gri) | L | well-known variety from Piedmont, producing wines of light color and a dry, tannic palate | 19/09 | 12/09 |
| Grisa rossa (gr) | L* | synonym for the French Grec rouge, once widely spread in many European regions for both table and wine, renowned for the beauty of its grapes tinged in rose and green | 19/09 | 12/09 |
| Malvasia moscata (mamo) | L | Muscat-flavored genotype, widely grown in Piedmont several centuries ago | 12/09 | 28/08 |
| Malvasia Schierano (ms) | L | aromatic genotype from central Piedmont, not grown elsewhere | 12/09 | 28/08 |
| Montanera (mp) | L | from the Alps, nearly extinct, has a remarkable enological potential | 05/09 | 05/09 |
| Moscato d'Amburgo (ma) | I | Muscat de Hambourg, renowned Muscat-flavored grape for table consumption | 12/09 | 21/08 |
| Moscato bianco (mobi) | I | Muscat à petits grains blancs, grown all over the world and widely cultivated in Piedmont for the production of the sparkling "Asti" | 05/09 | 21/08 |
| Moscato nero d'Acqui (mna) | L | aromatic variety from Piedmont grown today in a very limited extent | 19/09 | 12/09 |
| Nascetta (na) | L | ancient Piedmont genotype recently reassessed for the production of varietal quality wines | 12/09 | 12/09 |
| Nebbiolo (ne) | It | most reputed variety of the region, giving top-quality wines, including Barolo and Barbaresco, grown in Piedmont as well as in the Aosta valley and Valtellina | 19/09 | 05/09 |
| Nebue (nebue) | L | aromatic grape found in the alpine valley of Susa (Piedmont), currently nearly extinct | 05/09 | 21/08 |
| Neretto duro (nd) | L | early ripening vine spread in the past all over Piedmont for its generous yield, currently disappearing from modern vineyards | 05/09 | 21/08 |
| Pelaverga (cari) | L | local cultivar used for both table and wine production, giving pale, light-bodied wines | 19/09 | 12/09 |
| Pignola (p) | It | ancient variety nowadays hardly found in Valtellina and in the northern part of Piedmont | 19/09 | 05/09 |
| Pinot noir (pn) | I | | 05/09 | 21/08 |
| Ruché (ru) | L | aromatic cultivar from a restricted area near Asti, producing a peculiar, rose-scented dry wine | 12/09 | 12/09 |
| Teinturier (elliptic berry) (teb) | L* | deep red-fleshed, of obscure origin, once grown in marginal vineyards in Piedmont to add color to wines | 05/09 | 21/08 |
| Teinturier (round berry) (trb) | L* | red-fleshed grape, with small bunches of low sugar and neutral flavor, used as Teinturier in older vineyards | 05/09 | 21/08 |

[a]In the first column, the abbreviations used in the principal component analysis output is shown in parentheses. [b]L, local; L*, local with synonyms in other regions; It, Italian; I, international.

B ($CH_3OH$ 100%) were used to separate peaks, establishing a gradient between 5 and 100% of solvent B over 49 min at a flow rate of 0.48 mL $min^{-1}$. The DAD was set at an acquisition range of 200−700 nm. Flavonols were detected at 360 nm and HCTs at 320 nm. Flavonols were identified using pure standards (quercetin 3-*O*-glucopyranoside and myricetin 3-*O*-glucopyranoside) purchased from Extrasynthèse (Genay, France) and by analysis of the DAD spectrum and the retention time of each chromatographic peak with previously available data.[29]. All flavonols were read at 360 nm and the concentration of each flavonol was calculated through the external standard method. As each flavonol concentration was expressed as equivalents of quercetin 3-*O*-glucopyranoside, the concentrations of individual flavonols were multiplied by the ratio between their molecular weight and the molecular weight of quercetin 3-*O*-glucopyranoside.

HCT peaks were identified on the basis of their DAD spectra and retention times.[30] The *cis* and *trans* forms of *p*-coumaroyltartaric acid and the *trans* form of caffeoyltartaric acid were identified, together with lower amounts of *cis*-caffeoyltartaric acid as well as of *trans*-feruloyltartaric acid. HCTs were quantified as *p*-coumaric acid equivalents (as to *p*-coumaroyl- and caffeoyltartaric acids), and as ferulic acid equivalents (as to *trans*-feruloyltartaric acid), using external standards of *p*-coumaric and ferulic acids purchased from Fluka (Buchs, Switzerland). All HCTs were read at 320 nm; the concentration of each compound was calculated by the external standard

Table 2. Total Anthocyanin Concentrations and Anthocyanin Profiles (Percent) of the Skins of Some Colored Cultivars in Two Successive Years[a]

| | | total anth (mg kg$^{-1}$) | Df | Cy | Pt | Pn | Mv | acetyl | p-coum | caff | total free tri | total free di | total acyl | tri/di |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **pale-rose berry cultivars** | | | | | | | | | | | | | | |
| Barbarossa (from Piedmont) | 2006 | 55.1 | 0.5 | | 0.0 | 1.4 a | 0.1 | 0.0 | 0.2 b | 0.4 | 0.6 | 98.8 a | 0.6 | 0.0 |
| | 2007 | 57.2 | 0.8 | 96.9 | 0.2 | 0.4 b | 0.4 | 0.1 | 1.2 a | 0.1 | 1.4 | 97.2 b | 1.4 | 0.0 |
| Grisa rossa | 2006 | 26.3 | 0.6 | 97.1 | 0.1 | 0.4 | 0.7 | 0.2 | 0.8 | 0.0 | 1.5 | 97.6 | 1.0 | 0.0 |
| | 2007 | 32.2 | 1.6 | 95.2 | 1.2 | 0.8 | 0.2 | 0.2 | 0.8 | 0.0 | 3.0 | 96.0 | 1.0 | 0.0 |
| average | | 42.7 | 0.9 | 96.7 | 0.4 | 0.7 | 0.3 | 0.1 | 0.7 | 0.1 | 1.6 | 97.4 | 1.0 | 0.0 |
| **colored-berry cultivars** | | | | | | | | | | | | | | |
| Avanà | 2006 | 700.3 | 4.6 | 29.3 a | 4.4 a | 47.2 a | 12.0 | 0.2 | 2.3 | 0.0 | 21.0 b | 76.5 a | 2.5 | 0.28 b |
| | 2007 | 959.2 | 7.0 | 37.8 b | 6.0 b | 34.9 b | 11.6 | 0.4 | 2.4 | 0.0 | 24.6 a | 72.7 b | 2.7 | 0.34 a |
| Barbera | 2006 | 1219.7 | 14.9 b | 4.2 | 14.8 b | 3.4 | 41.9 a | 10.1 | 10.5 | 0.2 | 71.6 | 7.6 | 20.8 | 9.9 |
| | 2007 | 1264.2 | 19.6 a | 4.2 | 17.6 a | 3.0 | 34.6 b | 11.3 | 9.7 | 0.1 | 71.8 | 7.2 | 21.0 | 10.2 |
| Becuét | 2006 | 959.5 | 9.7 b | 1.4 | 9.5 b | 5.1 a | 40.8 a | 9.6 b | 23.2 | 0.7 a | 60.0 | 6.5 | 33.5 | 10.0 |
| | 2007 | 1082.0 | 11.4 a | 1.1 | 10.3 a | 3.6 b | 38.0 b | 12.9 a | 22.3 | 0.4 b | 59.7 | 4.7 | 35.6 | 13.3 |
| Brachetto | 2006 | 396.1 | 8.0 b | 6.0 a | 8.1 b | 25.9 a | 49.5 b | 0.4 | 1.9 b | 0.3 a | 65.7 b | 31.8 a | 2.5 | 2.1 b |
| | 2007 | 396.3 | 10.5 a | 4.3 b | 9.2 a | 14.8 b | 56.2 a | 0.5 | 4.2 a | 0.1 b | 75.9 a | 19.2 b | 4.9 | 3.7 a |
| Brachetto Roero | 2006 | 518.0 | 11.7 b | 29.7 b | 5.5 | 33.1 a | 11.1 a | 2.9 b | 5.9 | 0.0 | 28.4 | 62.8 | 8.8 | 0.4 |
| | 2007 | 579.6 | 14.8 a | 42.2 a | 5.8 | 21.6 b | 6.1 b | 3.3 a | 6.2 | 0.1 | 26.7 | 63.8 | 9.5 | 0.4 |
| Cabernet Sauvignon | 2006 | 1474.2 | 15.0 | 3.0 | 8.2 | 6.5 a | 35.0 a | 25.4 | 6.3 b | 0.6 a | 58.2 a | 9.6 b | 32.2 | 6.4 |
| | 2007 | 1379.8 | 15.7 | 4.1 | 8.2 | 8.6 b | 30.7 b | 24.7 | 7.8 a | 0.2 b | 54.6 b | 12.7 a | 32.7 | 4.6 |
| Pelaverga | 2006 | 410.7 | 20.2 | 25.1 | 10.0 | 22.8 | 18.3 | 0.4 a | 2.5 b | 0.7 a | 48.5 | 47.9 | 3.6 | 1.0 |
| | 2007 | 414.2 | 18.8 | 28.7 | 8.9 | 24.4 | 15.1 | 0.2 b | 3.8 a | 0.1 b | 42.8 | 53.1 | 4.0 | 0.8 |
| Croatina | 2006 | 1771.5 | 16.9 | 2.4 | 13.7 | 8.2 | 43.3 a | 7.1 b | 8.1 | 0.4 | 73.9 a | 10.6 | 15.6 | 7.2 |
| | 2007 | 1840.6 | 17.5 | 2.6 | 13.7 | 8.3 | 40.1 b | 8.3 a | 9.1 | 0.3 | 71.4 b | 10.9 | 17.8 | 6.7 |
| Dolcetto | 2006 | 1035.5 | 6.8 a | 0.9 a | 7.9 a | 6.3 a | 54.3 a | 6.0 | 16.7 b | 1.0 | 69.1 a | 7.3 a | 23.7 | 9.9 |
| | 2007 | 908.6 | 5.3 b | 0.5 b | 5.7 b | 3.9 b | 43.7 b | 7.8 | 32.2 a | 0.8 | 54.8 b | 4.4 b | 40.8 | 13.5 |
| Freisa | 2006 | 1664.9 | 8.2 | 17.2 b | 8.8 | 39.5 | 23.4 | 0.2 b | 2.6 | 0.0 b | 40.4 | 56.7 | 2.9 | 0.7 |
| | 2007 | 1602.7 | 8.8 | 21.6 a | 8.8 | 36.5 | 21.0 | 0.7 a | 2.5 | 0.1 a | 38.6 | 58.2 | 3.2 | 0.7 |
| Gambarossa | 2006 | 920.2 | 20.4 b | 10.3 | 10.6 b | 16.8 a | 36.4 | 1.1 | 4.2 | 0.1 b | 67.5 | 27.1 a | 5.4 | 2.5 b |
| | 2007 | 753.5 | 24.0 a | 10.2 | 11.9 a | 14.4 b | 32.6 | 2.5 | 4.3 | 0.2 a | 68.5 | 24.5 b | 6.9 | 2.8 a |
| Grignolino | 2006 | 407.5 | 3.6 | 13.9 | 2.6 b | 53.2 a | 19.8 b | 0.6 | 6.2 | 0.1 | 25.9 b | 67.1 a | 6.9 | 0.4 b |
| | 2007 | 457.6 | 4.8 | 12.4 | 4.2 a | 39.8 b | 31.6 a | 0.8 | 6.3 | 0.1 | 40.6 a | 52.2 b | 7.2 | 0.8 a |
| Malvasia Schierano | 2006 | 560.8 b | 17.5 b | 10.0 b | 15.8 b | 10.6 | 40.7 a | 0.4 | 3.9 | 1.0 a | 73.9 a | 20.6 b | 5.4 | 3.7 a |
| | 2007 | 799.4 a | 23.8 a | 14.1 a | 18.1 a | 11.5 | 28.3 b | 0.5 | 3.7 | 0.0 b | 70.2 b | 25.6 a | 4.2 | 2.6 b |
| Montanera | 2006 | 992.1 b | 12.2 | 2.2 | 8.8 | 6.7 a | 40.2 | 10.4 b | 19.2 | 0.3 | 61.2 | 9.0 | 29.8 | 7.4 |
| | 2007 | 1885.5 a | 12.5 | 1.5 | 10.2 | 4.9 b | 39.1 | 11.9 a | 19.6 | 0.3 | 61.8 | 6.4 | 31.9 | 9.8 |



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**Table 2. continued**

| | | total anth (mg kg$^{-1}$) | | Df | | Cy | | Pt | | Pn | | Mv | | acetyl | | p-coum | | caff | | total free tri | | total free di | | total acyl | | tri/di | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Moscato d'Amburgo | 2006 | 424.5 | | 5.4 | | 11.9 | b | 4.8 | | 50.8 | | 24.1 | a | 0.3 | a | 2.5 | | 0.2 | | 34.4 | | 62.7 | | 2.9 | | 0.6 | |
| | 2007 | 523.9 | | 7.1 | | 17.4 | a | 5.5 | | 48.7 | | 17.3 | b | 0.1 | b | 3.8 | | 0.1 | | 29.9 | | 66.0 | | 4.0 | | 0.4 | |
| Moscato nero d'Acqui | 2006 | 488.6 | b | 11.4 | b | 8.0 | b | 11.5 | b | 20.9 | | 42.8 | a | 0.7 | | 3.7 | | 1.1 | | 65.6 | a | 28.9 | b | 5.5 | | 2.3 | a |
| | 2007 | 592.0 | a | 14.0 | a | 12.6 | a | 12.4 | a | 20.2 | | 36.3 | b | 0.8 | | 3.6 | | 0.1 | | 62.7 | b | 32.8 | a | 4.5 | | 1.9 | b |
| Nebbiolo | 2006 | 667.9 | b | 6.6 | | 16.4 | b | 5.1 | | 44.2 | | 20.0 | a | 2.5 | b | 5.1 | | 0.2 | | 31.7 | | 60.6 | | 7.7 | | 0.5 | |
| | 2007 | 827.9 | a | 7.2 | | 22.1 | a | 5.2 | | 42.3 | | 14.1 | b | 4.5 | a | 4.5 | | 0.1 | | 26.5 | | 64.4 | | 9.1 | | 0.4 | |
| Nebue | 2006 | 2243.9 | | 14.8 | a | 3.6 | | 11.8 | | 10.3 | | 43.1 | | 7.3 | b | 8.7 | b | 0.4 | | 69.7 | a | 14.0 | | 16.4 | | 5.2 | |
| | 2007 | 1755.8 | | 11.9 | b | 2.5 | | 10.3 | | 8.6 | | 43.4 | | 9.5 | a | 13.4 | a | 0.4 | | 65.7 | b | 11.1 | | 23.3 | | 6.1 | |
| Neretto duro | 2006 | 1302.0 | | 17.2 | b | 5.5 | b | 16.2 | b | 3.9 | b | 36.2 | a | 9.2 | | 11.6 | | 0.1 | | 69.6 | a | 9.4 | b | 20.9 | | 7.5 | a |
| | 2007 | 1428.9 | | 19.8 | a | 8.7 | a | 17.1 | a | 4.8 | a | 28.7 | b | 9.3 | | 11.5 | | 0.1 | | 65.6 | b | 13.5 | a | 20.8 | | 4.8 | b |
| Pignola | 2006 | 522.8 | | 20.3 | | 22.0 | | 9.8 | | 19.4 | | 17.6 | | 5.9 | b | 4.7 | b | 0.2 | | 47.7 | | 41.4 | | 10.9 | | 1.2 | |
| | 2007 | 485.4 | | 20.5 | | 21.1 | | 9.6 | | 18.7 | | 15.9 | | 7.7 | a | 6.4 | a | 0.1 | | 46.1 | | 39.7 | | 14.2 | | 1.2 | |
| Pinot noir | 2006 | 726.3 | b | 6.6 | a | 3.4 | a | 8.2 | a | 27.6 | a | 54.2 | b | 0.0 | | 0.1 | | 0.0 | | 69.0 | b | 31.0 | a | 0.1 | | 2.2 | b |
| | 2007 | 1018.0 | a | 4.5 | b | 1.8 | b | 6.2 | b | 20.5 | b | 66.9 | a | 0.0 | | 0.0 | | 0.0 | | 77.7 | a | 22.3 | b | 0.0 | | 3.5 | a |
| Ruché | 2006 | 794.3 | | 6.7 | a | 1.7 | a | 7.5 | a | 13.3 | | 59.6 | | 1.2 | b | 9.3 | b | 0.6 | | 73.9 | | 15.0 | | 11.1 | | 5.0 | |
| | 2007 | 783.4 | | 4.8 | b | 1.0 | b | 5.8 | b | 11.7 | | 61.7 | | 2.1 | a | 12.1 | a | 0.7 | | 72.3 | | 12.7 | | 15.0 | | 5.8 | |
| average | | 953.2 | | 12.3 | | 11.4 | | 9.4 | | 20.0 | | 33.6 | | 5.0 | | 7.9 | | 0.3 | | 55.3 | | 31.4 | | 13.2 | | 4.1 | |
| **red-fleshed cultivars** | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Alicante Bouschet | 2006 | 1826.1 | b | 4.4 | b | 1.3 | b | 5.1 | b | 22.8 | | 44.8 | | 1.9 | b | 19.2 | | 0.5 | a | 54.2 | | 24.1 | | 21.6 | | 2.3 | |
| | 2007 | 2296.1 | a | 6.4 | a | 1.6 | a | 7.3 | a | 19.9 | | 43.7 | | 3.0 | a | 17.9 | | 0.2 | b | 57.4 | | 21.6 | | 21.0 | | 2.7 | |
| Teinturier (elliptical berry) | 2006 | 4672.2 | | 18.3 | | 3.1 | b | 12.4 | b | 7.8 | b | 36.8 | a | 15.5 | b | 6.0 | | 0.1 | | 67.5 | a | 10.9 | b | 21.6 | | 6.6 | a |
| | 2007 | 4698.9 | | 19.5 | | 4.1 | a | 14.4 | a | 9.2 | a | 29.7 | b | 16.8 | a | 6.2 | | 0.1 | | 63.6 | b | 13.3 | a | 23.1 | | 5.0 | b |
| Teinturier (round berry) | 2006 | 2821.2 | a | 7.8 | | 2.8 | a | 11.2 | a | 7.6 | a | 42.8 | | 8.9 | b | 18.7 | b | 0.2 | b | 61.8 | a | 10.4 | a | 27.8 | | 6.1 | b |
| | 2007 | 2703.7 | b | 6.3 | | 1.4 | b | 8.4 | b | 5.8 | b | 40.3 | | 9.9 | a | 27.3 | a | 0.4 | a | 55.1 | b | 7.2 | b | 37.7 | | 7.7 | a |
| average | | 3147.7 | | 14.2 | | 4.3 | | 13.0 | | 10.4 | | 32.9 | | 11.7 | | 13.4 | | 0.2 | | 60.1 | | 14.7 | | 25.2 | | 4.9 | |
| year | | ns | | ** | | *** | | ns | | ** | | ** | | ns | | ** | | ns | | ns | | ns | | ns | | ns | |
| cultivar | | *** | | *** | | *** | | *** | | *** | | *** | | *** | | *** | | ns | | *** | | *** | | *** | | *** | |
| interaction year × cultivar | | *** | | *** | | *** | | ns | | *** | | *** | | *** | | *** | | ns | | ** | | ** | | *** | | *** | |

[a]For each variety, means followed by different letters are significantly different for $P \leq 0.05$. Significance of year, cultivar, and interaction year × cultivar effects was tested for $P \leq 0.05$ (*), $P \leq 0.01$ (**), and $P \leq 0.0001$ (***); ns = not significant. Df = delphinidin 3-*O*-glucoside; Cy = cyanidin 3-*O*-glucoside; Pt = petunidin 3-*O*-glucoside; Pn = peonidin 3-*O*-glucoside; Mv = malvidin 3-*O*-glucoside; acetyl = sum of the percentages of acetylglucosides; p-coum = sum of the percentages of *p*-coumaroylglucosides; caff = sum of the percentages of caffeoylglucosides; total free tri = sum of the percentages of nonacylated trihydroxylated anthocyanins; total free di = sum of the percentages of nonacylated dihydroxylated anthocyanins.



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method, and results were multiplied by the ratio between the molecular weight of each compound and the molecular weight of *p*-coumaric acid for *p*-coumaroyl and caffeoyl derivatives and of ferulic acid for feruloyl derivatives.

The sum of individual flavonols and HCTs was calculated to express the respective totals as milligrams per kilogram of fresh berries.

**Statistical Analysis.** Data were subjected to analysis of variance (ANOVA) separating means by the Duncan's test at $P \leq 0.05$; the significance of years, cultivars, and their interaction was also calculated. The interaction between cultivars and years was evaluated by calculating the least-squares means (LS means) selecting $P \leq 0.0001$, $P \leq 0.01$, and $P \leq 0.05$ for significance of comparisons. Normalized (average = 0, variance = 1) data were submitted to principal component analysis (PCA) with the aim of discriminating cultivars on the basis of the studied variable association. All statistics were performed with SAS 8.2 for Windows (SAS Institute, Cary, NC, USA).

## RESULTS

**Anthocyanins (Table 2).** Total skin anthocyanin amounts ranged from 26 to 57 mg $kg^{-1}$ berry weight in cultivars with pale-rose berries, from 396 to 2244 in cultivars with colored berries, and from 1826 to 4699 in red-fleshed cultivars. The accumulation of total anthocyanin was significantly year-dependent only in 7 cultivars (Malvasia di Schierano, Montanera, Moscato nero d'Acqui, Nebbiolo, Pinot noir, Alicante Bouschet, and Teinturier round berry) of the 34 studied. As expected, the cultivar and the interaction year × cultivar, but not the year, significantly ($P < 0.0001$) affected total anthocyanin concentrations.

Among the free forms of anthocyanins, only the percentage of petunidin 3-*O*-glucoside was not year-dependent, but as variations within tri- and dihydroxylated anthocyanins compensated, the ratio between the two forms of anthocyanin was not influenced by the year, whereas, as expected, it was largely dependent on the genotype. The stability of this parameter over the years makes it a good tool for chemotaxonomic purposes, as previously proposed.[31] In colored-berry cultivars, the tri/dihydroxylated anthocyanin ratio ranged between 0.3 and 13.5, and it ranged between 2.3 and 7.7 in red-fleshed cultivars. In pale-rose berry cultivars, trihydroxylated anthocyanins were nearly absent, their anthocyanins profile being characterized by a net prevalence of cyanidin 3-*O*-glucoside (Table 2).

The percentage of total acylated anthocyanins was very low (<1.4%) in pale-rose cultivars, whereas it ranged between 2.5 and 40.8% in colored-berry cultivars (Pinot noir excluded) and between 21.0 and 37.7% in red-flashed cultivars. Acetyl and caffeoyl derivatives of anthocyanins were not significantly affected by the yearly climatic conditions, whereas the percentages of *p*-coumaroyl derivatives and of total acylated forms were vintage-dependent. Acylation with *p*-coumaric acid was predominant, except in French Cabernet Sauvignon (as also shown in ref 31) and Teinturier elliptic berry and in the Italian Pignola. In Barbera and Croatina, the percentages of acetyl and *p*-coumaroyl derivatives were similar. Acylation with caffeic acid was very rare, with a relative incidence not higher than 1.1% (Table 2). Acylation was lower in the cooler 2006 with respect to 2007 (Table 2) in accordance with the authors of ref 32, who assessed that acylated anthocyanin derivatives decreased when the climatic region became cooler.

**Flavonols (Tables 3 and 4).** Among flavonols, the analytical method we used allowed us to identify the main grape flavonols: myricetin 3-*O*-glucoside, quercetin 3-*O*-glucoside, quercetin 3-*O*-glucuronide, kaempferol 3-*O*-glucoside, and kaempferol 3-*O*-glucuronide. According to data available in the literature,[14] where the flavonol profile of 64 red varieties and 27 white varieties was described, these flavonols account for 86% of total flavonols in red varieties and for 98% in white varieties.

The vintage effect was marked on flavonol concentrations, which were significantly lower in 2006 as compared to 2007 (when the vegetative season was characterized by higher solar irradiation). Only in a few cultivars was the total flavonol accumulation not significantly influenced by vintage (Gambarossa, Nebbiolo, Teinturier round berry, and the white Nascetta).

The total amount of flavonols in the skins of colored-berry cultivars ranged from 21.7 mg $kg^{-1}$ (Dolcetto) to 175.8 mg $kg^{-1}$ (Teinturier round berry) of berry weight in 2006, whereas in 2007 it ranged from 78.6 mg $kg^{-1}$ (Dolcetto) to 297.9 mg $kg^{-1}$ (Nebue) (Table 3). Noncolored cultivars showed values between 32 mg $kg^{-1}$ (in Cortese and Malvasia moscata) and >100 mg $kg^{-1}$ in Nascetta (Table 4). In colored grapes the accumulation of flavonols was on average 1.8 times higher than in white berries. However, some white cultivars were able to accumulate quantities of flavonols comparable to or even higher than those of colored genotypes; in particular, the cultivar Nascetta accumulated considerable amounts of flavonols in both years (123.2 mg $kg^{-1}$ in 2006 and 167.8 mg $kg^{-1}$ in 2007).

The main flavonol compounds present in berry skins were quercetin 3-*O*-glucoside and quercetin-3-*O*-glucuronide (about 75% in total across all genotypes and years), the first being more abundant than the second in both years in 28 (23 with colored and 5 with noncolored berries) of 34 studied genotypes. In colored-berry cultivars the vintage significantly affected the percentage of total quercetin glycosides, whereas in white-berry cultivars the sum of the quercetin glycosides was not vintage-dependent (Tables 3 and 4). The ratio between the quercetin glycosides (glucoside/glucuronide) was similar in the different coloration groups; it was anyway significantly affected by the cultivar and by the vintage (it was higher when total flavonol concentration was lower).

As expected, no myricetin 3-*O*-glucoside was detected in white cultivars except trace amounts in Chasselas in 2007 (accounting for 0.26% of flavonol total amount, data not shown). The percentage of myricetin 3-*O*-glucoside was close to zero in pale-rose berry genotypes; in colored-berry cultivars it ranged from 2.2 to 49.7% in 2006 and from 1.7 to 28% in 2007 (average throughout both years = 15.8%) and was on average higher in red-fleshed cultivars (33.4%). The percentage of myricetin 3-*O*-glucoside was generally significantly influenced by the year (Table 3).

In colored-berry cultivars, kaempferol was mostly present as glucoside in both years. In 2006, kaempferol 3-*O*-glucuronide was generally not detected, whereas in 2007 its relative abundance ranged from nil to 6.2% in Moscato nero d'Acqui; in several cultivars, namely, Cabernet Sauvignon, Dolcetto, Freisa, Grignolino, and Pinot noir, it was never detected (Table 3).

**HCTs (Tables 5 and 6).** Among HCTs, we identified *trans*-caffeoyltartaric acid, *cis*- and *trans*-*p*-coumaroyltartaric acids, and *trans*-feruloyltartaric acid. The total skin concentration of HCTs ranged from 16.6 mg $kg^{-1}$ (Moscato d'Amburgo) to 115.1 mg $kg^{-1}$ (Gambarossa) in 2006 and from 18.7 mg $kg^{-1}$ (Nebbiolo) to 125.7 mg $kg^{-1}$ (Nebue) in 2007. The total concentrations of HCTs and the percentages of individual HCTs were not affected by the vintage, except that of feruloyltartaric acid in both colored- and white-berry cultivars. The main HCTs were *trans*-caffeoyltartaric acid, *trans*-*p*-coumaroyltartaric acid, and *cis*-coumaroyltartaric acid. A net negative correlation was found between the *p*-coumaroyltartaric

Table 3. Total Flavonol Concentrations and Flavonol Profiles (Percent) of the Skin of the Colored Grape Cultivars in Two Successive Years[a]

| | | total flav (mg kg$^{-1}$) | | Myr 3OG | | Q 3Ogl | | Q 3OG | | K 3Ogl | | K 3OG | | total Qs | | total Ks | | Q 3OG/Q 3Ogl | | Myr/Qs | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **pale-rose berry cultivars** | | | | | | | | | | | | | | | | | | | | | |
| Barbarossa (from Piedmont) | 2006 | 142.8 | b | 0.7 | | 32.6 | | 50.4 | | 0.2 | b | 16.1 | a | 83.0 | | 16.3 | | 1.5 | | 0.01 | |
| | 2007 | 212.9 | a | 0.7 | | 33.3 | | 50.8 | | 2.6 | a | 12.6 | b | 84.1 | | 15.2 | | 1.5 | | 0.01 | |
| Grisa rossa | 2006 | 60.9 | | 0.0 | | 36.6 | a | 41.6 | | 0.0 | b | 21.8 | | 78.2 | a | 21.8 | b | 1.1 | | 0.00 | |
| | 2007 | 91.4 | | 0.0 | | 30.3 | b | 42.9 | | 2.5 | a | 24.3 | | 73.2 | b | 26.8 | a | 1.4 | | 0.00 | |
| average | | 127.0 | | 0.4 | | 33.2 | | 46.4 | | 1.3 | | 18.7 | | 79.6 | | 20.0 | | 1.4 | | 0.0044 | |
| **colored-berry cultivars** | | | | | | | | | | | | | | | | | | | | | |
| Avanà | 2006 | 29.1 | b | 5.1 | a | 43.3 | | 45.3 | | 0.0 | b | 6.2 | | 88.7 | | 6.2 | b | 1.0 | | 0.06 | a |
| | 2007 | 162.9 | a | 2.4 | b | 37.0 | | 45.4 | | 4.6 | a | 10.7 | | 82.4 | | 15.2 | a | 1.2 | | 0.03 | b |
| Barbera | 2006 | 104.7 | b | 28.1 | a | 26.8 | b | 36.1 | | 0.0 | b | 9.1 | a | 62.9 | b | 9.1 | | 1.3 | a | 0.45 | a |
| | 2007 | 151.7 | a | 19.6 | b | 34.0 | a | 36.9 | | 2.2 | a | 7.3 | b | 70.9 | a | 9.5 | | 1.1 | b | 0.28 | b |
| Becuét | 2006 | 36.1 | b | 49.7 | a | 15.4 | b | 20.8 | b | 0.0 | b | 14.1 | a | 36.2 | b | 14.1 | | 1.3 | a | 1.37 | a |
| | 2007 | 112.3 | a | 20.8 | b | 35.6 | a | 30.3 | a | 2.7 | a | 10.6 | b | 66.0 | a | 13.3 | | 0.9 | b | 0.31 | b |
| Brachetto | 2006 | 33.9 | b | 10.2 | | 33.0 | | 47.1 | | 0.0 | b | 9.7 | | 80.1 | a | 9.7 | b | 1.4 | | 0.13 | |
| | 2007 | 121.1 | a | 8.3 | | 30.7 | | 46.8 | | 3.0 | a | 11.3 | | 77.4 | b | 14.2 | a | 1.5 | | 0.11 | |
| Brachetto Roero | 2006 | 53.2 | b | 4.0 | a | 24.0 | | 50.9 | a | 0.0 | b | 21.1 | | 74.8 | | 21.1 | b | 2.1 | | 0.05 | a |
| | 2007 | 208.7 | a | 1.7 | b | 24.0 | | 48.4 | b | 3.3 | a | 22.6 | | 72.4 | | 25.9 | a | 2.0 | | 0.02 | b |
| Cabernet Sauvignon | 2006 | 104.5 | b | 30.0 | a | 18.4 | b | 34.3 | | 0.0 | | 17.3 | a | 52.7 | b | 17.3 | a | 1.9 | a | 0.57 | a |
| | 2007 | 131.2 | a | 21.6 | b | 31.6 | a | 35.0 | | 0.0 | | 11.7 | b | 66.7 | a | 11.7 | b | 1.1 | b | 0.32 | b |
| Pelaverga | 2006 | 42.6 | b | 5.2 | | 24.8 | | 55.9 | | 0.0 | b | 14.1 | | 80.7 | | 14.1 | | 2.3 | | 0.06 | |
| | 2007 | 91.6 | a | 3.7 | | 28.5 | | 47.9 | | 5.3 | a | 14.6 | | 76.4 | | 19.9 | | 1.7 | | 0.05 | |
| Croatina | 2006 | 93.4 | b | 38.0 | a | 16.5 | b | 27.5 | b | 0.0 | | 18.0 | | 44.0 | b | 18.0 | | 1.7 | | 0.86 | a |
| | 2007 | 183.0 | a | 28.0 | b | 22.3 | a | 32.0 | a | 1.9 | | 15.8 | | 54.3 | a | 17.7 | | 1.4 | | 0.52 | b |
| Dolcetto | 2006 | 21.7 | b | 40.9 | a | 18.5 | b | 22.1 | b | 0.0 | | 18.6 | a | 40.5 | b | 18.6 | a | 1.2 | | 1.01 | a |
| | 2007 | 78.6 | a | 21.7 | b | 37.7 | a | 32.0 | a | 0.0 | | 8.7 | b | 69.6 | a | 8.7 | b | 0.8 | | 0.31 | b |
| Freisa | 2006 | 73.4 | b | 7.8 | a | 22.7 | | 62.3 | | 0.0 | | 7.2 | | 85.0 | | 7.2 | | 2.7 | | 0.09 | a |
| | 2007 | 161.0 | a | 5.6 | b | 25.8 | | 58.7 | | 0.0 | | 9.8 | | 84.5 | | 9.8 | | 2.3 | | 0.07 | b |
| Gambarossa | 2006 | 125.2 | | 13.9 | | 30.1 | | 38.3 | a | 0.6 | b | 17.1 | | 68.4 | a | 17.7 | | 1.3 | | 0.20 | |
| | 2007 | 185.0 | | 13.2 | | 30.3 | | 35.5 | b | 5.3 | a | 15.7 | | 65.8 | b | 21.0 | | 1.2 | | 0.20 | |
| Grignolino | 2006 | 110.2 | b | 2.2 | | 20.8 | b | 61.6 | | 0.0 | | 15.5 | a | 82.3 | b | 15.5 | a | 3.0 | | 0.03 | |
| | 2007 | 186.2 | a | 2.4 | | 25.5 | a | 58.9 | | 0.0 | | 13.2 | b | 84.4 | a | 13.2 | b | 2.3 | | 0.03 | |
| Malvasia Schierano | 2006 | 47.3 | b | 18.1 | a | 32.6 | | 41.3 | | 0.0 | b | 8.1 | b | 73.8 | | 8.1 | b | 1.3 | | 0.24 | |
| | 2007 | 144.2 | a | 10.2 | b | 35.2 | | 40.8 | | 4.2 | a | 9.6 | a | 76.0 | | 13.8 | a | 1.2 | | 0.13 | |
| Montanera | 2006 | 57.1 | b | 36.2 | b | 22.8 | | 27.7 | b | 0.0 | b | 13.3 | | 50.5 | b | 13.3 | | 1.2 | | 0.72 | a |
| | 2007 | 179.0 | a | 23.2 | a | 27.6 | | 34.8 | a | 2.1 | a | 12.3 | | 62.4 | a | 14.4 | | 1.3 | | 0.37 | b |
| Moscato d'Amburgo | 2006 | 22.9 | b | 6.9 | a | 38.3 | | 46.3 | a | 0.0 | | 8.5 | | 84.6 | | 8.5 | | 1.2 | a | 0.08 | |
| | 2007 | 100.9 | a | 3.4 | b | 45.1 | | 37.7 | b | 4.7 | | 9.1 | | 82.7 | | 13.8 | | 0.8 | b | 0.04 | |
| Moscato nero d'Acqui | 2006 | 52.8 | b | 15.8 | | 42.8 | a | 31.1 | b | 0.7 | b | 9.6 | b | 73.9 | | 10.3 | b | 0.7 | b | 0.21 | a |
| | 2007 | 169.4 | a | 8.3 | | 35.4 | b | 37.3 | a | 6.2 | a | 12.8 | a | 72.7 | | 19.0 | a | 1.1 | a | 0.11 | b |
| Nebbiolo | 2006 | 91.4 | | 4.0 | | 21.4 | | 57.2 | b | 0.0 | b | 17.5 | a | 78.6 | b | 17.5 | a | 2.7 | | 0.05 | |
| | 2007 | 138.7 | | 3.9 | | 21.1 | | 61.9 | a | 1.8 | a | 11.2 | b | 83.0 | a | 13.0 | b | 2.9 | | 0.05 | |
| Nebue | 2006 | 82.1 | b | 23.4 | a | 38.7 | | 27.0 | b | 0.3 | | 10.5 | | 65.7 | b | 10.8 | | 0.7 | | 0.36 | a |



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**Table 3. continued**

| | | total flav (mg kg$^{-1}$) | | Myr 3OG | | Q 3Ogl | | Q 3OG | | K 3Ogl | | K 3OG | | total Qs | | total Ks | | Q 3OG/Q 3Ogl | | Myr/Qs | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2007 | 297.9 | a | 14.4 | b | 38.2 | | 32.2 | a | 2.5 | | 12.7 | | 70.4 | a | 15.2 | | 0.8 | | 0.20 | b |
| Neretto duro | 2006 | 54.7 | b | 39.7 | a | 37.9 | | 16.5 | b | 0.0 | b | 5.8 | | 54.5 | b | 5.8 | b | 0.4 | b | 0.73 | a |
| | 2007 | 208.8 | a | 21.3 | b | 38.1 | | 30.2 | a | 3.1 | a | 7.3 | | 68.3 | a | 10.4 | a | 0.8 | a | 0.31 | b |
| Pignola | 2006 | 112.8 | b | 8.6 | a | 15.6 | | 61.4 | | 0.0 | b | 14.4 | | 77.0 | | 14.4 | b | 3.9 | | 0.11 | a |
| | 2007 | 178.1 | a | 5.9 | b | 18.0 | | 60.0 | | 3.1 | a | 13.1 | | 78.0 | | 16.1 | a | 3.3 | | 0.08 | b |
| Pinot noir | 2006 | 24.9 | b | 16.2 | a | 35.8 | | 33.2 | | 0.0 | | 14.8 | a | 69.0 | b | 14.8 | a | 0.9 | | 0.24 | a |
| | 2007 | 81.3 | a | 13.7 | b | 40.1 | | 35.3 | | 0.0 | | 10.9 | b | 75.4 | a | 10.9 | b | 0.9 | | 0.18 | b |
| Ruché | 2006 | 93.2 | b | 22.9 | a | 27.2 | | 31.3 | b | 0.2 | b | 18.5 | | 58.5 | | 18.6 | | 1.2 | | 0.39 | a |
| | 2007 | 158.4 | a | 16.8 | b | 27.6 | | 34.6 | a | 3.0 | a | 18.0 | | 58.0 | | 22.6 | | 1.3 | | 0.27 | b |
| average | | 111.3 | | 15.8 | | 29.5 | | 40.6 | | 1.4 | | 12.7 | | 70.0 | | 14.1 | | 1.5 | | 0.3 | |
| **red-fleshed cultivars** | | | | | | | | | | | | | | | | | | | | | |
| Alicante Bouschet | 2006 | 52.5 | b | 30.4 | a | 13.5 | b | 32.3 | | 0.0 | b | 23.7 | | 45.9 | b | 23.7 | | 2.4 | a | 0.66 | a |
| | 2007 | 162.0 | a | 20.2 | b | 23.2 | a | 34.0 | | 4.2 | a | 18.5 | | 57.1 | a | 22.7 | | 1.5 | b | 0.35 | b |
| Teinturier (elliptical berry) | 2006 | 87.0 | b | 50.9 | a | 16.1 | b | 20.3 | b | 0.0 | | 12.6 | a | 36.5 | b | 12.6 | a | 1.3 | | 1.40 | a |
| | 2007 | 257.7 | a | 31.3 | b | 31.1 | a | 29.5 | a | 0.0 | | 8.2 | b | 60.6 | a | 8.2 | b | 1.0 | | 0.52 | b |
| Teinturier (round berry) | 2006 | 175.8 | | 41.3 | a | 19.3 | b | 26.6 | | 0.0 | b | 12.7 | a | 46.0 | b | 12.7 | | 1.4 | | 0.90 | a |
| | 2007 | 248.7 | | 26.2 | b | 29.8 | a | 31.4 | | 2.6 | a | 10.0 | b | 61.2 | a | 12.6 | | 1.1 | | 0.43 | b |
| average | | 157.1 | | 36.6 | | 21.6 | | 28.8 | | 0.8 | | 12.2 | | 50.4 | | 13.0 | | 1.4 | | 0.8 | |
| year | | *** | | ** | | ** | | ns | | *** | | ns | | ** | | ns | | ** | | * | |
| cultivar | | *** | | *** | | *** | | *** | | *** | | *** | | *** | | *** | | *** | | *** | |
| interaction year × cultivar | | *** | | *** | | *** | | *** | | *** | | *** | | *** | | *** | | *** | | *** | |

[a]For each variety, means followed by different letters are significantly different for $P \leq 0.05$. Significance of year, cultivar, and interaction year × cultivar effects was tested for $P \leq 0.05$ (*), $P \leq 0.01$ (**), and $P \leq 0.0001$ (***); ns = not significant. Myr 3OG = myricetin 3-*O*-glucoside; Q 3Ogl = quercetin 3-*O*-glucuronide; Q 3OG = quercetin 3-*O*-glucoside; K 3Ogl = kaempferol 3-*O*-glucuronide; K 3OG = kaempferol 3-*O*-glucoside; total Qs = sum of quercetin glycosides; total Ks = sum of kaempferol glycosides; Myr/Qs = ratio myricetin 3-*O*-glucoside/sum of quercetin glycosides.



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**Table 4. Total Flavonol Concentrations and Flavonol Profiles (Percent) of the Skins of the White Grape Cultivars in Two Successive Years[a]**

| | | total flav (mg kg$^{-1}$) | | Q 3Ogl | | Q 3OG | | K 3Ogl | | K 3OG | | Q 3Ogl + Q 3OG | | K 3Ogl + K 3OG | | Q 3OG/ Q 3Ogl | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Arneis | 2006 | 99.6 | b | 19.9 | b | 61.4 | a | 0.0 | b | 18.7 | a | 81.3 | | 18.7 | | 3.1 | a |
| | 2007 | 154.1 | a | 29.6 | a | 52.4 | b | 2.3 | a | 15.7 | b | 82.0 | | 18.0 | | 1.8 | b |
| Chardonnay | 2006 | 39.1 | b | 21.0 | b | 53.4 | a | 0.0 | b | 25.6 | a | 74.4 | b | 25.6 | a | 2.5 | a |
| | 2007 | 112.3 | a | 31.9 | a | 47.9 | b | 2.4 | a | 17.8 | b | 79.8 | a | 20.2 | b | 1.5 | b |
| Chasselas blanc | 2006 | 40.7 | b | 34.2 | | 54.9 | a | 0.0 | b | 10.9 | b | 89.1 | a | 10.9 | b | 1.6 | a |
| | 2007 | 126.5 | a | 37.9 | | 46.8 | b | 2.5 | a | 12.5 | a | 84.7 | b | 15.0 | a | 1.2 | b |
| Cortese | 2006 | 32.9 | b | 24.9 | | 62.3 | a | 0.0 | b | 12.8 | | 87.2 | | 12.8 | | 2.5 | |
| | 2007 | 60.6 | a | 30.0 | | 53.9 | b | 4.0 | a | 12.2 | | 83.9 | | 16.1 | | 1.8 | |
| Malvasia moscata | 2006 | 32.4 | b | 48.9 | | 41.5 | | 3.4 | b | 9.6 | | 90.4 | | 9.3 | | 0.9 | |
| | 2007 | 69.2 | a | 46.5 | | 40.7 | | 0.0 | a | 9.3 | | 87.2 | | 13.0 | | 0.9 | |
| Moscato bianco | 2006 | 48.9 | b | 41.1 | | 44.3 | a | 0.0 | b | 14.6 | | 85.4 | a | 14.6 | b | 1.1 | |
| | 2007 | 142.3 | a | 40.0 | | 37.7 | b | 4.4 | a | 17.8 | | 77.8 | b | 22.2 | a | 0.9 | |
| Nascetta | 2006 | 123.2 | | 15.9 | b | 40.9 | b | 0.1 | | 43.1 | a | 56.7 | b | 43.2 | a | 2.6 | |
| | 2007 | 167.8 | | 20.0 | a | 52.0 | a | 1.6 | | 26.4 | b | 72.0 | a | 28.0 | b | 2.6 | |
| average | | 89.3 | | 31.6 | | 49.3 | | 1.5 | | 17.6 | | 80.8 | | 19.1 | | 1.8 | |
| year | | *** | | ns | | ns | | *** | | ns | | ns | | ns | | * | |
| cultivar | | *** | | *** | | *** | | *** | | *** | | *** | | *** | | *** | |
| interaction year × cultivar | | * | | *** | | *** | | *** | | *** | | *** | | *** | | *** | |

[a]For each variety, means followed by different letters are significantly different for $P \leq 0.05$. Significance of year, cultivar, and interaction year × cultivar effects was tested for $P \leq 0.05$ (*), $P \leq 0.01$ (**), and $P \leq 0.0001$ (***); ns = not significant. Q 3Ogl = quercetin 3-*O*-glucuronide; Q 3OG = quercetin 3-*O*-glucoside; K 3Ogl = kaempferol 3-*O*-glucuronide; K 3OG = kaempferol 3-*O*-glucoside; sum of Qs = sum of quercetin glycosides; sum of Ks = sum of kaempferol glycosides.

acids and *trans*-caffeoyltartaric acid concentrations (Pearson correlation coefficient was −0.98, $P \leq 0.0001$). In colored-grape cultivars the ratio between the sum of *p*-coumaroyltartaric acids and *trans*-caffeoyltartaric acid was always >1, except in Gambarossa, Moscato d'Amburgo, Pinot noir, and Teinturier elliptical berry (Table 5). *trans*-Feruloyltartaric acid content was generally very low or nil; a few cultivars (Freisa, Nebbiolo, and Pignola) did not accumulate this compound at all (Table 5). No correlation was observed between the percentage of total *p*-coumaroylated HCTs (on total HCTs) and the percentage of *p*-coumaroylated anthocyanins on total anthocyanins ($R^2$ = 0.0028, ns).

In white cultivars, HCT contents ranged between 24 and 98 mg kg$^{-1}$ and the relationships between specific HCT compounds were similar to those observed for colored cultivars. However, among these white genotypes, Cortese and Nascetta showed a net prevalence of caffeoyltartaric acid over *p*-coumaroyltartaric (Table 6).

**Discrimination of Cultivars Based on Their Polyphenol Profiles.** We tested the capacity of flavonols and HCTs to discriminate *V. vinifera* cultivars, independently from their skin color, by performing principal component analyses (PCAs) with these two classes of compounds. A first PCA was done exclusively on flavonols (using as variables only percentage compositions as total concentrations were highly year-dependent). The six variables used (average percentages of the two years) were the percentages of myricetin 3-*O*-glucoside, quercetin 3-*O*-glucuronide, and 3-*O*-glucoside, the sum of quercetins, the sum of kaempferols, and the ratio between the quercetin forms. On the first principal component (PRIN1) we found myricetin 3-*O*-glucoside, quercetin 3-*O*-glucoside, and the sum of quercetins; on the second principal component (PRIN2) we found quercetin 3-*O*-glucuronide. The first two principal components accounted for 86% of total variance. Total kaempferol was included in the third PRIN, and it was able alone to justify a further 14% of the total variance. The results showed that quercetin 3-*O*-glucoside and myricetin 3-*O*-glucoside efficiently discriminated cultivars (Figure 1) and were negatively correlated with each other ($R$ = −0.82), confirming that *V. vinifera* cultivars can be classified according to the prevalence of one of these two flavonols.[14,15,33] Quercetin 3-*O*-glucuronide contributed to the separation of individuals on PRIN2; cv. Nebue in particular was characterized by a very high percentage of quercetin 3-*O*-glucuronide over total flavonols (Figure 1). Nascetta, in the three-dimensional plot of individuals, was well distinguished from the other cultivars due to its association with the third PRIN, that is, its high quantities of kaempferol.

Next, we performed a PCA with five variables (we used average values of the two years as the year effect was absent or extremely low, as shown in Table 5) associated with the HCT metabolism (the four HCT individual percentages and total HCT concentration). Opposite loadings on PRIN1 for caffeoyltartaric acid and *p*-coumaroyltartaric acid (correlation coefficient $R$ = −0.98) were noted; these same two compounds were associated with PRIN1, whereas total HCTs with PRIN2. The total variance explained by the first two PRINs was 78%. Similarly to the two main flavonols, the two main HCTs were able to distinguish cultivars; individuals associated with the negative values of PRIN1 were characterized by low percentages of *p*-coumaroyltartaric acid (between 10 and 32%) and high percentages of caffeoyltartaric acid (Figure 2).

The discriminatory capacity of flavonols and HCTs together with that of anthocyanins was finally tested in colored cultivars through a PCA performed on 15 variables, including exclusively profile data (Table 7). Performing PCA on normalized averages of the two separate years resulted in PCA models in which individuals studied in the two years were generally close in the $x-y$ plane, implying that the PCA models obtained in the two


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Table 5. Total Hydroxycinnamates and HCT Profiles (Percent) of the Colored Grape Cultivars in Two Successive Years[a]

| | | total HCTs (mg kg$^{-1}$) | | trans CT | | cis p-coumT | | trans p-coumT | | trans fT | | pcum/CT | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **pale-rose berry cultivars** | | | | | | | | | | | | | |
| Barbarossa (from Piedmont) | 2006 | 40.7 | a | 49.7 | a | 11.5 | b | 38.3 | b | 0.5 | | 1.0 | b |
| | 2007 | 36.3 | b | 42.0 | b | 13.5 | a | 43.8 | a | 0.7 | | 1.4 | a |
| Grisa roussa | 2006 | 38.2 | | 47.1 | | 13.7 | | 39.1 | | 0.0 | b | 1.1 | |
| | 2007 | 29.1 | | 56.5 | | 13.0 | | 29.7 | | 0.7 | a | 0.8 | |
| average | | 36.1 | | 48.8 | | 12.9 | | 37.7 | | 0.5 | | 1.1 | |
| **colored-berry cultivars** | | | | | | | | | | | | | |
| Avanà | 2006 | 31.1 | | 31.3 | a | 13.0 | a | 55.4 | b | 0.3 | | 2.2 | b |
| | 2007 | 33.5 | | 27.6 | b | 10.5 | b | 61.4 | a | 0.5 | | 2.6 | a |
| Barbera | 2006 | 74.0 | | 43.4 | a | 6.8 | | 49.2 | b | 1.6 | a | 1.3 | b |
| | 2007 | 78.9 | | 38.5 | b | 7.2 | | 53.5 | a | 0.8 | b | 1.6 | a |
| Becuét | 2006 | 36.7 | b | 49.6 | a | 6.9 | | 40.8 | b | 2.8 | | 1.0 | b |
| | 2007 | 47.7 | a | 44.4 | b | 7.0 | | 46.0 | a | 2.6 | | 1.2 | a |
| Brachetto | 2006 | 29.6 | b | 38.6 | b | 12.8 | a | 48.0 | a | 0.6 | a | 1.6 | a |
| | 2007 | 51.9 | a | 44.8 | a | 9.7 | b | 45.2 | b | 0.3 | b | 1.2 | b |
| Brachetto Roero | 2006 | 70.8 | | 48.3 | a | 7.9 | | 43.0 | b | 0.8 | b | 1.0 | a |
| | 2007 | 66.0 | | 44.9 | b | 7.9 | | 45.9 | a | 1.3 | a | 1.2 | a |
| Cabernet Sauvignon | 2006 | 38.8 | | 46.9 | a | 9.7 | b | 41.4 | b | 2.4 | | 1.1 | b |
| | 2007 | 44.2 | | 43.0 | b | 7.0 | a | 48.0 | a | 2.0 | | 1.3 | a |
| Pelaverga | 2006 | 30.3 | b | 23.2 | | 16.7 | | 60.0 | | 0.3 | | 3.3 | |
| | 2007 | 25.8 | a | 23.2 | | 16.0 | | 60.1 | | 0.7 | | 3.3 | |
| Croatina | 2006 | 86.6 | a | 44.2 | a | 7.1 | | 47.6 | | 1.2 | b | 1.2 | b |
| | 2007 | 46.1 | b | 38.3 | b | 6.6 | | 51.3 | | 3.8 | a | 1.5 | a |
| Dolcetto | 2006 | 30.3 | b | 39.9 | a | 8.3 | a | 50.0 | b | 1.7 | b | 1.5 | b |
| | 2007 | 36.4 | a | 33.6 | b | 6.6 | b | 52.6 | a | 7.2 | a | 1.8 | a |
| Freisa | 2006 | 17.8 | b | 22.1 | | 12.1 | | 65.6 | b | 0.0 | | 3.6 | |
| | 2007 | 26.5 | a | 20.7 | | 11.7 | | 67.6 | a | 0.0 | | 3.8 | |
| Gambarossa | 2006 | 115.1 | | 78.4 | a | 2.2 | b | 18.6 | b | 0.7 | | 0.3 | b |
| | 2007 | 94.6 | | 71.2 | b | 3.7 | a | 24.2 | a | 0.9 | | 0.4 | a |
| Grignolino | 2006 | 65.4 | a | 37.8 | b | 9.3 | | 53.0 | | 0.0 | b | 1.6 | |
| | 2007 | 40.9 | b | 38.9 | a | 8.6 | | 51.7 | | 0.8 | a | 1.6 | |
| Malvasia di Schierano | 2006 | 74.2 | a | 24.4 | b | 10.3 | | 65.3 | a | 0.0 | | 3.1 | a |
| | 2007 | 55.6 | b | 26.4 | a | 8.8 | | 64.1 | b | 0.7 | | 2.8 | b |
| Montanera | 2006 | 58.7 | | 28.2 | | 8.9 | | 60.0 | | 2.8 | b | 2.4 | |
| | 2007 | 54.4 | | 26.1 | | 8.4 | | 60.5 | | 4.9 | a | 2.6 | |
| Moscato d'Amburgo | 2006 | 16.6 | b | 38.5 | | 5.1 | | 20.0 | b | 1.3 | | 0.3 | |
| | 2007 | 34.3 | a | 69.3 | | 4.7 | | 24.4 | a | 1.5 | | 0.4 | |
| Moscato nero d'Acqui | 2006 | 53.9 | | 36.9 | | 10.4 | | 52.7 | b | 0.0 | b | 1.7 | |
| | 2007 | 59.4 | | 34.4 | | 9.3 | | 55.8 | a | 0.5 | a | 1.9 | |
| Nebbiolo | 2006 | 25.8 | a | 27.0 | | 12.7 | | 60.3 | | 0.0 | | 2.7 | |
| | 2007 | 18.7 | b | 28.5 | | 12.8 | | 58.6 | | 0.0 | | 2.5 | |
| Nebue | 2006 | 101.5 | b | 28.4 | | 6.9 | a | 64.2 | | 0.5 | b | 2.5 | |
| | 2007 | 125.3 | a | 27.5 | | 5.4 | b | 66.3 | | 0.8 | a | 2.6 | |
| Neretto duro | 2006 | 40.1 | | 44.1 | a | 5.9 | b | 47.3 | | 2.6 | a | 1.2 | b |
| | 2007 | 43.8 | | 41.8 | b | 6.9 | a | 49.8 | a | 1.6 | b | 1.4 | a |
| Pignolo | 2006 | 41.0 | a | 28.5 | b | 11.0 | | 59.9 | b | 0.0 | | 2.5 | a |
| | 2007 | 28.0 | b | 31.1 | a | 6.4 | | 62.5 | | 0.0 | | 2.2 | b |
| Pinot noir | 2006 | 26.1 | b | 55.6 | a | 4.9 | b | 38.9 | | 0.7 | b | 0.8 | |
| | 2007 | 40.7 | a | 49.2 | b | 7.8 | a | 41.3 | | 1.7 | a | 1.0 | |
| Ruchè | 2006 | 41.2 | a | 30.7 | | 11.1 | | 56.8 | | 1.4 | b | 2.2 | |
| | 2007 | 24.6 | b | 30.6 | | 9.7 | | 54.8 | | 4.6 | a | 2.1 | |
| average | | 48.9 | | 38.8 | | 8.9 | | 50.3 | | 1.3 | | 1.8 | |
| **Teinturier cultivars** | | | | | | | | | | | | | |
| Alicante Bouschet | 2006 | 115.0 | | 44.0 | | 5.2 | | 47.9 | | 2.7 | | 1.2 | |
| | 2007 | 102.3 | | 44.3 | | 4.2 | | 49.2 | | 2.2 | | 1.2 | |
| Teinturié (elliptical berry) | 2006 | 65.0 | | 51.9 | b | 3.6 | b | 40.7 | | 3.7 | | 0.8 | a |
| | 2007 | 68.9 | | 56.3 | a | 4.8 | a | 36.4 | b | 2.5 | | 0.7 | b |


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**Table 5. continued**

| | | total HCTs (mg kg$^{-1}$) | trans CT | cis p-coumT | trans p-coumT | trans fT | pcum/CT |
|---|---|---|---|---|---|---|---|
| Teinturié (round berry) | 2006 | 63.6 | 64.1 a | 2.4 | 30.0 a | 5.2 b | 0.5 b |
| | 2007 | 57.8 | 57.2 b | 2.3 | 29.3 | 11.2 a | 0.6 a |
| average | | 54.0 | 41.5 | 8.2 | 48.1 | 1.6 | 1.6 |
| average 2006 | | 54.3 | 43.3 | 8.7 | 46.8 | 1.2 b | 1.6 |
| average 2007 | | 51.5 | 41.9 | 8.2 | 47.9 | 1.9 a | 1.6 |
| year | | ns | ns | ns | ns | * | ns |
| cultivar | | *** | *** | *** | *** | *** | *** |
| interaction year × cultivar | | *** | *** | *** | ** | *** | *** |

[a]For each variety means followed by different letters are significantly different for $P \leq 0.05$. Significance of year, cultivar, and interaction year × cultivar effects was tested for $P \leq 0.05$ (*), $P \leq 0.01$ (**), and $P \leq 0.0001$ (***); ns = not significant. trans CT = *trans*-caffeoyltartaric acid; cis p-coumT = *cis*-*p*-coumaroyltartaric acid; trans p-coumT = *trans*-*p*-coumaroyltartaric acid; trans fT = *trans*-feruloyltartaric acid; p-coum/CT = ratio *p*-coumaroyltartaric acid (*cis* + *trans*)-caffeoyltartaric acid.

**Table 6. Total Hydroxycinnamates and HCT Profiles (Percent) of the White Grape Cultivars in Two Successive Years[a]**

| | | total HCTs (mg kg$^{-1}$) | trans CT | cis p-coumT | trans p-coumT | trans fT | pcoum/CT |
|---|---|---|---|---|---|---|---|
| Arneis | 2006 | 60.5 | 26.9 a | 12.5 | 60.6 b | 0.0 b | 2.8 b |
| | 2007 | 58.5 | 19.9 b | 13.0 | 66.3 a | 0.8 a | 4.0 a |
| Chardonnay | 2006 | 48.1 | 53.5 a | 9.1 | 36.9 b | 0.4 | 0.9 b |
| | 2007 | 53.1 | 46.8 b | 9.5 | 43.2 a | 0.6 | 1.1 a |
| Chasselas blanc | 2006 | 52.9 | 34.3 a | 12.9 | 50.4 | 2.4 b | 1.8 b |
| | 2007 | 53.7 | 27.2 b | 14.8 | 51.3 | 6.7 a | 2.4 a |
| Cortese | 2006 | 27.9 | 87.8 a | 2.4 | 8.8 b | 1.0 | 0.1 b |
| | 2007 | 23.9 | 83.0 b | 4.0 | 11.1 a | 1.9 | 0.2 a |
| Malvasia moscata | 2006 | 35.0 | 24.3 | 13.0 | 62.7 | 0.0 b | 3.1 |
| | 2007 | 33.9 | 24.9 | 13.9 | 60.2 | 0.9 a | 3.0 |
| Moscato bianco | 2006 | 87.3 | 26.7 b | 12.8 a | 60.5 a | 0.0 b | 2.8 a |
| | 2007 | 98.4 | 32.4 a | 9.9 b | 56.9 b | 0.8 a | 2.1 b |
| Nascetta | 2006 | 93.0 a | 63.1 a | 5.4 b | 30.24 b | 1.3 | 0.6 b |
| | 2007 | 73.4 b | 57.5 b | 7.5 a | 33.7 a | 1.3 | 0.7 a |
| average | | 57.1 | 43.5 | 10.1 | 45.2 | 1.3 | 1.8 |
| year | | ns | ns | ns | ns | * | ns |
| cultivar | | *** | *** | *** | *** | *** | *** |
| interaction year × cultivar | | *** | *** | *** | *** | *** | *** |

[a]For each variety, means followed by different letters are significantly different for $P \leq 0.05$. Significance of year, cultivar, and interaction year × cultivar effects was tested for $P \leq 0.05$ (*), $P \leq 0.01$ (**), and $P \leq 0.0001$ (***); ns = not significant. trans CT = *trans*-caffeoyltartaric acid; cis p-coumT = *cis*-*p*-coumaroyltartaric acid; trans p-coumT = *trans*-*p*-coumaroyltartaric acid; trans fT = *trans*-feruloyltartaric acid; p-coum/CT = ratio *p*-coumaroyltartaric acid (*cis* + *trans*)-caffeoyltartaric acid.

different years were similar; that is, PRINs were built with the same variables. For this reason we decided to average the data of the two years to gain clarity in the output display. The model proposed (Table 7) justified 68% of total variance with the first three PRINs. According to the eigenvalues, five variables (namely, the percentages of myricetin 3-*O*-glucoside, quercetin 3-*O*-glucoside, *p*-coumaroyl anthocyanin derivatives, *trans*-feruloyltartaric acid, and malvidin 3-*O*-glucoside) were associated with PRIN1. On PRIN2 we found variables associated with the hydroxycinnamate metabolism, namely, the percentages of caffeoyltartaric acid, on the one hand, and of *p*-coumaroyltartaric acid, on the other; as expected and already discussed, these two variables were negatively correlated with each other. Quercetin 3-*O*-glucuronide was negatively associated with the third principal component (PRIN3). Individuals located on the positive part of the PRIN2 axis (Figure 3) were rich in *p*-coumaroyl tartrates (at least 70% of total concentration) and vice versa for individuals located in the opposite side of the axis. Individuals localized in the upper and positive part of the *z*-axis (PRIN3) were low in quercetin 3-*O*-glucuronide (Figure 3).

## DISCUSSION

Due to a worldwide and long history of cultivation, several thousands of grape cultivars exist, which represent a wealth of metabolic diversity, partly exploited today but still very promising for the future. Characterization of this diversity is important to (a) provide new genotypes for quality winemaking and for health protection purposes; (b) design enological techniques adapted to specific cultivars; (c) draw hypotheses

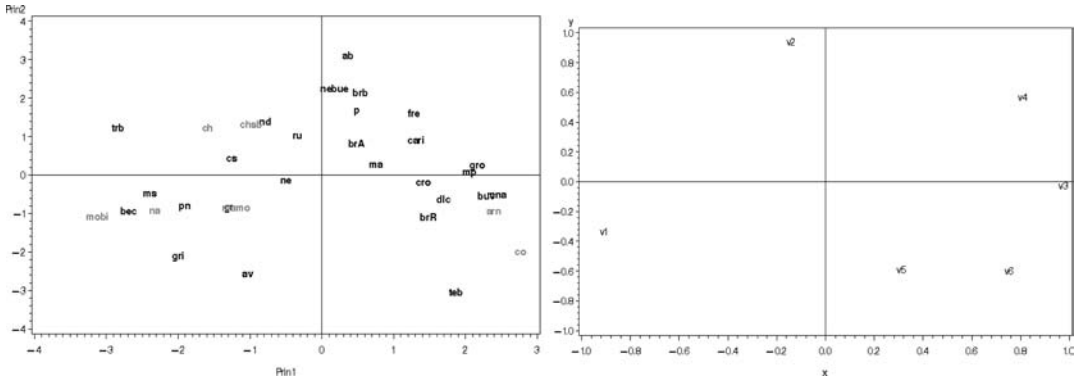

**Figure 1.** Bidimensional distribution of individuals and of variables according to a PCA model using flavonol profile data (averages of the two years of trial). Acronyms of white berry skin cultivars are reported in gray. See Table 1 for variety identification. V1 = % of myricetin 3-*O*-glucoside, V2 = % of quercetin 3-*O*-glucuronide, V3 = % of quercetin 3-*O*-glucoside, V4 = sum of quercetin percentages, V5 = sum of kaempferol percentages, V6 = ratio between quercetin glucoside and glucuronide.

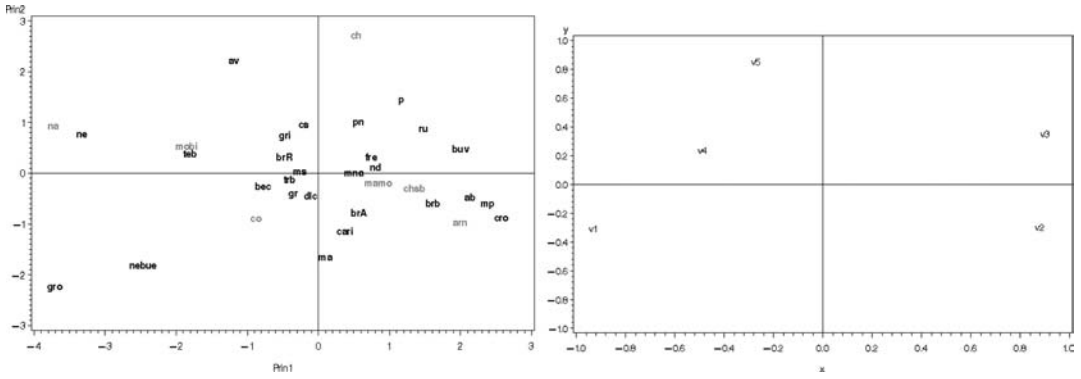

**Figure 2.** Bidimensional distribution of individuals and of variables according to a PCA model using HCT profiles and concentrations (averages of the two years of trial). Acronyms of white berry skin cultivars are reported in gray. See Table 1 for variety identification. V1 = % of *trans*-caffeoyltartaric acid, V2 = % of *cis*-*p*-coumaroyltartaric acid, V3 = % of *trans*-*p*-coumaroyltartaric acid, V4 = % of *trans*-feruloyltartaric acid, V5 = HCT total concentration.

**Table 7. Eigenvectors of the Examined Variables on the Three Principal Components (PRIN1, PRIN2, and PRIN3)[a]**

| | PRIN1 | PRIN2 | PRIN3 |
|---|---|---|---|
| *trans*-caffeoyltartaric acid | 0.06 | **−0.52** | −0.33 |
| *p*-coumaroyltartaric acid (*trans* + *cis* forms) | −0.10 | **0.53** | 0.29 |
| *trans*-feruloyltartaric acid | **0.32** | −0.25 | 0.18 |
| myricetin 3-*O*-glucoside | **0.41** | −0.02 | 0.002 |
| quercetin 3-*O*-glucuronide | −0.07 | −0.06 | **−0.38** |
| quercetin 3-*O*-glucoside | **−0.36** | 0.16 | 0.09 |
| sum of kaempferols | −0.09 | −0.27 | 0.36 |
| anth acetyl-derivatives | 0.29 | −0.02 | −0.04 |
| anth *p*-coumaroyl derivatives | **0.32** | −0.08 | 0.33 |
| anth caffeoyl derivatives | 0.22 | 0.11 | 0.37 |
| delphinidin 3-*O*-glucoside | 0.16 | 0.30 | −0.33 |
| cyanidin 3-*O*-glucoside | −0.298 | −0.22 | −0.09 |
| petunidin 3-*O*-glucoside | 0.27 | 0.32 | −0.34 |
| peonidin 3-*O*-glucoside | −0.25 | −0.10 | −0.08 |
| malvidin 3-*O*-glucoside | **0.30** | 0.08 | 0.008 |
| | | | |
| eigenvalues | 5.56 | 2.56 | 1.94 |
| total variance | 0.37 | 0.18 | 0.13 |

[a]Eigenvalues of the three PRINs and their contribution to total variance. In bold letters are the variables associated with the appropriate PRIN.

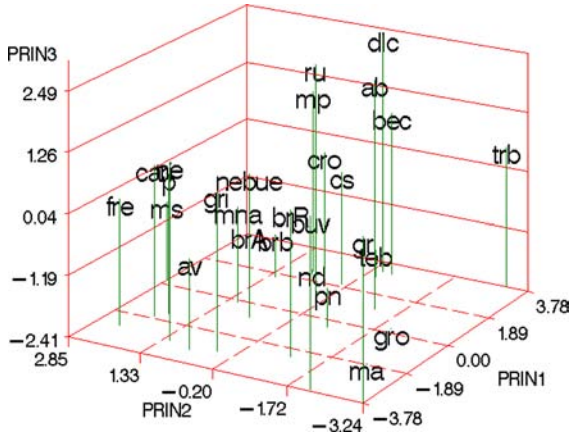


**Figure 3.** Three-dimensional distribution of individuals (exclusively colored grape cultivars) according to a PCA model using anthocyanin, flavonol, and HCT profiles. See Table 1 for variety identification.

on the biosynthetic pathways underlying fruit composition; (d) provide chemotaxonomic models to be used in the study of genetic relationships and to help in the assessment of the varietal composition of musts and, potentially, of wines.

To contribute to this characterization, in this study we analyzed the fruit skin phenolic composition of 34 grape genotypes across two years: most of these genotypes are minor cultivars that could be exploited in the future for their particular characteristics. As expected, we observed a large diversity in


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polyphenolic composition of berry skins of these genotypes, involving both the colored compounds and other phenolic classes (flavonols, HCTs) that contribute to the wine technological properties and to the health-promoting properties of grapes.

**Possible Implications of HCT Diversity on Winemaking Techniques.** It is well-known that different grape cultivars are characterized by specific anthocyanin and flavonol profiles, which bear basic importance in the determination of wine properties, in particular, color intensity and hue. In the vinification process of colored grapes, the cultivars rich in 3′-hydroxylated anthocyanins are generally penalized because these pigments, preferentially extracted during the initial phase of maceration, may be easily oxidized by the enzymes present in the juice.[4] Cultivars having anthocyanin profiles dominated by trihydroxylated molecules are instead more protected against oxidation.[34] The extent of anthocyanin acylation is also important for enological purposes, as acylated anthocyanins are more stable than the free forms and are more effective in color stabilization of wines.[31,34,35]

In this study we show for the first time that, besides anthocyanins and flavonols, also the HCT pattern is very diverse in grape genotypes, being alternatively dominated by *p*-coumaric and caffeic derivatives. This diversity can potentially have a major impact on winemaking, as HCTs have pivotal roles in the evolution of color and browning of wines. In the vinification of white grapes, enzymatic oxidation, starting as soon as the grapes are crushed, results in degradation of phenolic compounds and browning. The first step leading to browning is the enzymatic oxidation of caffeoyltartrate and *p*-coumaroyltartrates, which are the major substrates of polyphenol oxidase, to *o*-quinones, and the intensity of browning depends on their concentration.[36] In wine, HCT contents decrease during aging with a parallel increase in oxidative browning (absorbance at 420 nm).[37] The intensity of browning phenomena is mainly related to *cis*- and *trans*-caffeoyltartaric acid content, which depends on the variety.[37] Consequently, the wines produced by Nascetta, Moscato bianco, and Chardonnay, the grapes of which contained higher concentrations of *trans*-caffeoyltartaric acid (50, 28, and 25 mg $kg^{-1}$, respectively, as averages of the two years) could be more susceptible to browning during vinification and shelf life. These hypotheses find confirmation in the literature: when blends of grapes containing Chardonnay were used during Cava sparkling wine production, they underwent browning more often than musts subjected to the same processes but without Chardonnay grapes.[37] The use of solid $CO_2$ (cryomaceration) during vinification increases the concentration of HCTs in the wine because of low grape polyphenol oxidase activity, induced by the lower oxygen level present in the must.[38]

HCTs are also linked to off-odor appearance in wines, particularly in red wines during aging in wood. Namely, the formation of volatile phenols by *Brettanomyces/Dekkera* yeast is the result of enzymatic transformation of grape HCTs, as the action of enzymes with cinnamoylesterase activity releases these weak acids as their free forms, which are then decarboxylated into hydroxystyrenes and reduced into their corresponding ethyl derivative forms (4-ethylphenol, 4-ethylxguaiacol, and 4-ethylcatechol).[39] The formation of volatile phenols in wine is proportional both to the size of the *Brettanomyces/Dekkera* populations and to the concentration of their precursors in grapes.[39] Therefore, red wines produced by cultivars such as Croatina, Barbera, Gambarossa, and Nebue, characterized by higher concentrations of HCTs, could be penalized in wineries with *Brettanomyces/Dekkera* contamination. High contents of HCTs were also detected in red-fleshed cultivars, in particular, in Alicante Bouschet grapes, which, however, are never elaborated in purity.

**Biosynthesis of Acylated Anthocyanins and HCTs.** Metabolomic analysis across different genotypes can yield clues on the biosynthetic pathways leading to specific compounds,[14] and this is of particular interest for ill-defined or yet unknown biosynthetic pathways, as is the case for anthocyanin acylation, flavonol glucuronylation, and HCT biosynthesis.

The acylation step of anthocyanins has been studied in different plants but is still obscure in grape. Anthocyanin acyltransferases (AATs) have been isolated in a few plants and are part of the BAHD subfamily of acyltransferases.[40] Reported AATs of different species act equally well on different anthocyanidin substrates.[41] This is indirectly supported by our data in the case of grape, as the incidence of single free anthocyanidins on total free anthocyanins was very close to the incidence of the respective acylated forms on total acylated anthocyanins across all colored cultivars (e.g., in the case of malvidin, these two measures showed a significant correlation with $R^2 = 0.92$). On the contrary, reported AATs are specific to either aliphatic (acetyl and malonyl) or aromatic (caffeoyl, coumaroyl, sinapoyl, and feruloyl) acyl-CoA.[41] The ratios between concentrations of acetylated (aliphatic) and total aromatic acyl glucosides were relatively constant for each genotype across vintages, but they displayed differences among cultivars, most of them showing an aliphatic/aromatic ratio below 1, whereas four of them (Barbera, Cabernet Sauvignon, Pignola, and Teinturier elliptical berry) had ratios higher than 1 in at least one season. No genotypes lacked one only of the two classes of acyl glucosides. The more straightforward explanation of these data is the existence in grape of different AATs, respectively specific to aliphatic and aromatic acyl-CoA, with different expression levels in different genotypes. The putative aromatic AAT would have a clear preference for *p*-coumarate above caffeate as suggested by the low abundance of the latter type of anthocyanin acylation.

3-*O*-Glycosylation is a constant characteristic of anthocyanin and flavonols in plants, and the glycosyl decoration differs in the number and type of sugar moieties, so further contributing to the diversity of these molecules. In grapevine, glycosylation patterns are simpler than in other plants, 3-*O*-glucosylation being the most common. The functional properties and expression patterns of the UDP-glucose flavonoid glucosyltransferase (UFGT) gene of *V. vinifera* have been well characterized.[17,42] The recombinant protein from this gene accepts flavonol in addition to anthocyanidin aglycones, albeit with a 50 times lower activity; however, the biosynthesis of flavonols starts before véraison, whereas UFGT is expressed only after this ripening stage. This opens the possibility that flavonols are glucosylated by a specific enzyme. Although anthocyanins in grape are constantly glucosylated, flavonols are also glucuronylated.[14−16] In our survey glucuronides were a very minor part of kaempferol glycosides, but represented about 40% of glycosides of quercetin. Our compositional data suggest that an UDP-glucuronate transferase acting on flavonols should have an expression pattern concentrated in the period before véraison, when myricetin is not yet produced due to the lack of F3′5′ expression,[8] and should have a preference for quercetin above kaempferol. A UDP-glucuronyltransferase acting on flavonols (VvGT5) in the grape berry skin has been recently described.[43] Consistent with our results, expression of this gene is high

already before véraison, and the recombinant enzyme shows a preference for quercetin above kaempferol.[43]

Biosynthesis of hydroxycinnamates in grapevine has not been detailed yet. Whereas in other plants esters of hydoxycinnamic acids with different acids (tartaric, quinic, shikimic) are present, in grape skins only tartrate esters have been found. Two pathways, possibly operating in different plants, can synthesize hydroxycinnamate esters. In the first pathway, hydroxycinnamoyl moieties are transferred to acceptor acids from CoA esters;[24,25] in the second pathway, organic acids are activated by glycosylation, and the glycosides are transesterified by hydroxycinnamic acids.[44,45] The only enzyme involved in the biosynthetic pathway of hydroxycinnamates that up to now has shown the ability to accept tartrate is an aromatic acyltransferase of *Equisetum arvense*.[46] This enzyme follows the first pattern, transferring hydroxycinnamoyl residues from CoA onto tartaric acid, and has a clear preference for caffeoyl- and coumaroyl-CoA above other hydroxycinnamoyl-CoA. A similar enzyme could be active in grape berry skins, as hydroxycinnamate biosynthesis in grape has high preference for *p*-coumaric and caffeic acid and only side activity for ferulic acid.

**Discrimination of Grape Cultivars Based on Flavonoid Profiles.** The use of metabolic analysis for recognition of grape cultivars has been pursued since HPLC techniques have been available, as they potentially offer the possibility to prove the presence of a specific variety in wine, where DNA is hardly detected due to nucleic acid degradation during winemaking. Although metabolites such as phenols are affected by factors such as environment and seasonal variations, polyphenolic profiles (i.e., the relative amounts of each compound) are rather stable in grapes, allowing the discrimination of single or groups of cultivars. In colored cultivars, anthocyanins offer easy and largely described chemotaxonomical opportunities.[28,47] Flavonols have recently been used for chemometrics and have been shown to be able to discriminate cultivars, too.[14,15,33] These studies proved that colored berry skin *V. vinifera* cultivars can be classified on the basis of the prevalence of di- or trihydroxylated anthocyanins and flavonols, namely, cyanidin and malvidin 3-*O*-glucosides, on the one hand, and myricetin and quercetin 3-*O*-glucosides, on the other. Besides, we show that colored-skin cultivars are also discriminated according to the pattern of anthocyanin acylation, confirming previous results.[47,48] Moreover, in this study we show that HCTs are another class of phenolic compounds, accumulating in both colored and white cultivars, which could be effective in *V. vinifera* cultivar classification. The two main HCTs, namely, caffeoyltartrate and *p*-coumaroyltartrate, allowed variety separation upon PCA. The discriminating potential of HCTs is high: as a matter of fact, when HCT variables were used in a PCA together with anthocyanins and flavonols, the second principal component was exclusively associated with caffeoyltartrate and *p*-coumaroyltartrates, thus showing their power in variety discrimination. Interestingly, quercetin 3-*O*-glucuronide allowed a further level of discrimination, justifying a residual 13% of variance on the third principal component. The use of HCTs as discrimination tools among cultivars is particularly appealing for noncolored grapes, for which classification based on anthocyanins is not possible, possibly together with other discriminating compound present in these grapes, such as flavonols.[14]

This work shows how a more global approach to the study of *V. vinifera* phenolic metabolites can improve the method of classifying cultivars. Further studies, possibly including proanthocyanidins and flavor-associated compounds, could improve classification tools and could deepen our knowledge about the biosynthetic pathways of grape secondary metabolism compounds.

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