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Profiling of Hydroxycinnamoyl Tartrates and of 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 as concerns major compounds (non-acylated anthocyanins and flavonols), but much less as regards 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 North-western 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.

We discuss the results of this study 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 coloured grapes), flavonols, flavan-3-ols, proanthocyanidins, and in non-flavonoid phenols such as hydroxycinnamoyl tartrates (HCTs). Flavonol and HCT concentrations are second to proanthocyanidins and to anthocyanidins in berry skins, while in berry pulps, apart from anthocyanin-containing red-fleshed grapes, HCTs are considered 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 colour and perform complex interactions with other phenolic substances under oxidative conditions during winemaking and wine ageing (*4, 5*). The biosynthesis of anthocyanidins and their glycosylation pathways are relatively well known, (*6, 7*), and a few genes which 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 coloured grapes. From a biological point of view, their role seems to be linked to UV screening (*10*) and, technologically, they are involved in the colour stabilization of red wines, through co-pigmentation 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 di-hydroxylated quercetin and the tri-hydroxylated myricetin, but other compounds such as the mono-hydroxylated kaempferol, and the methylated isorhamnetin, laricitrin and syringetin have also been identified (*13, 14*). Two recent comprehensive works by Castillo-Muñoz and co-workers (*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 at a lesser extent to *O*-methylation. In grape, hydroxylases and a methyltransferase which 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 non-flavonoid phenols in grapes and wines. The predominant HCTs in *V. vinifera* grape berry pulps and skins are caffeoyltartaric (caftaric) acid, *p*-coumaroyltartaric (coutaric) acid and feroulyltartaric (fertaric) acid, whose *trans* isomers 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 mg L^{-1} to several hundreds mg L^{-1} (*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 taxonomy of young single-variety wines (22). Besides, they take part in the formation of derived pigments with anthocyanins and contribute to colour stabilization in aging wines (23). The biosynthetic pathway of HCTs in grapevine is not known, while 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 which can help to single out specific genotypes, to be used both for basic studies and to assess 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 non-acylated anthocyanins and flavonols, while very few studies on a wide genotype range of *Vitis vinifera* have been performed as regards 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 *Vitis vinifera* cultivars over a period of two years. Among the studied genotypes, seven had non-coloured berries, two had pale-rose berries, 22 had coloured 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 *Vitis 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 to September) was cooler than the corresponding period in 2007 (the summation of daily average temperatures >10°C was 1893°C 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, whose berry phenol composition had not been analyzed in detail yet; three major Italian cultivars (Barbera, Dolcetto, Nebbiolo), and seven international cultivars (Cabernet sauvignon, Chardonnay, Chasselas blanc, Moscato bianco = White muscat, Moscato d'Amburgo = Muscat of Hambourg, Alicante Bouschet, Pinot noir) (Tab. 1). Vines were trained to a vertical trellis system and Guyot pruned. Canopies were routinely managed during spring and summer accordingly to the standard cultural practices of the cultivation area. In addition, crop load was controlled and standardized with cluster removal in the pre-véraison period. For each variety and in both years, berries were collected when they had reached a total soluble solid content of 20 ± 1 °Brix.

In the vineyard every cultivar was present as duplicate plots of 10 to 20 vines. After a preliminary measurement of soluble solid performed directly in the vineyard on ten berries per plot for each cultivar, if the SSC was 19 ± 1°Brix, about 25 berries from each plot were collected for each cultivar, from the upper, the middle and the bottom parts of the clusters and the shaded and exposed sides of the row, and pooled together. The soluble solid content of twenty berries was measured again in the lab and only if both measurements (the one in the vineyard and the one in the lab) ranged from 19 to 21 °Brix the remaining collected berries were divided in three sub-groups 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 ten-berry samples were processed as described by (*26*). Briefly, skins were manually separated from seeds and pulps, and extracted in a pH 3.2 ethanol buffer containing 2g/L of $Na_2S_2O_5$ at 30 °C for 72 hours.

Analysis of anthocyanins

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

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 x 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). Non-acylated anthocyanins were identified by comparison with pure standards purchased from Extrasynthèse (Genay, France), when available. The remaining anthocyanins were identified by matching DAD spectrum and retention time of each chromatographic peak with available data in literature (28). The percentages of individual anthocyanins were determined comparing the area of the individual peak with the total peak area.

Analysis of flavonols and HCTs

The ten-berry skin extract was diluted 1.1 fold with phosphoric acid 1 M. Extracts were filtered through 0.2 µm GHP Membrane Filters (Pall Corporation, 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 x 0.4 cm ID) column with a LiChrocart C18 guard column (Merck, Darmstadt, Germany). As previously reported (*26)* solvent A (phosphoric acid 10^{-3} M) and solvent B (CH_3OH 100%) were used to separate peaks, establishing a gradient between 5% and 100% of solvent B over 49 minutes 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, concentration 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*-feroulyltartaric acid. HCTs were quantified as *p*-coumaric acid equivalents (as to *p*-coumaroyl and caffeoyl tartaric acids), and as ferulic acid equivalents (as to *trans*-feroulyltartaric 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 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 mg kg^{-1} 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 Square Means (LS means) selecting $P \leq \cdot 0.0001$, $P \leq \cdot 0.01$ 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, 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 coloured 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) out of 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 di-hydroxylated 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 coloured-berry cultivars, the tri/di-hydroxylated 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, tri-hydroxylated 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 coloured-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 by 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 respect to 2007 (Table 2) in accordance with (32), who assessed that acylated anthocyanin derivatives decreased when the climatic region became cooler.

Flavonols (Table 3 and Table 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 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 the total flavonol accumulation was not significantly influenced by vintage (Gambarossa, Nebbiolo, Teinturier round berry and in the white Nascetta).

The total amount of flavonols in the skins of coloured-berry cultivars ranged from 21.7 (Dolcetto) to 175.8 (Teinturier round berry) mg kg^{-1} of berry weight in 2006, whereas in 2007 it ranged from 78.6 (Dolcetto) to 297.9 mg kg^{-1} (Nebue) (Table 3). Non-coloured cultivars showed values between 32 mg kg^{-1} (in Cortese and Malvasia moscata) and values higher than 100 mg kg^{-1} in Nascetta (Table 4). In coloured grapes the accumulation of flavonols was in the average 1.8 times higher than in white berries. However, some white cultivars were able to accumulate quantities of flavonols comparable or even higher than those of coloured 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 coloured and 5 with non-coloured berries) out of 34 studied genotypes. In coloured-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 coloured-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 in the average higher in red-flashed cultivars (33.4%). The percentage of myricetin 3-*O*-glucoside was generally significantly influenced by the year (Table 3).

In coloured 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).

Hydroxycinnamoyl tartrates (HCTs) (Table 5 and Table 6)

Among HCTs, we identified *trans* caffeoyltartaric acid, *cis* and *trans* *p*-coumaroyltartaric acids, and *trans*-feroulyltartaric 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 HCT were not affected by the vintage, except that of feroulyltartaric acid in both coloured 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 acids and *trans*-caffeoyl tartaric acid concentrations (Pearson correlation coefficient was -0.98, $P \leq 0.0001$). In coloured-grape cultivars the ratio between the sum of *p*-coumaroyltartaric acids and *trans* caffeoyltartaric acid was always higher than 1, except in Gambarossa, Moscato d'Amburgo, Pinot noir and in Teinturier elliptic berry (Table 5). *Trans* feroulyltartaric 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 that observed for coloured 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 *Vitis vinifera* cultivars, independently of their skin colour, 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 lied on 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 (Fig. 1) and were negatively correlated with each other (R = -0.82), confirming that *Vitis 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 (Fig. 1). Nascetta, in the three-dimension plot of individuals, was well distinguished from the other cultivars due to its association to the third PRIN, i.e. to its high quantities of kaempferol.

Next, we performed a PCA with 5 variables (we used average values of the two years as the year effect was absent or extremely low, as shown in Table 5) associated to 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 noticed; these same two compounds were associated to PRIN1 whereas total HCTs to 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 to the negative values of

PRIN1 were characterized by low percentages of p-coumaroyl tartaric acid (between 10 and 32 %) and high percentages of caffeoyl tartaric acid (Fig. 2).

The discriminatory capacity of flavonols and HCTs together with that of anthocyanins was finally tested in coloured cultivars through a PCA performed on 15 variables, including exclusively profile data (Tab. 7). Performing PCA on normalized averages of the two separate years resulted in PCA models where individuals studied in the two years were generally close in the x-y plane, implying that the PCA models obtained in the two different years were similar, i.e. PRINs were built with the same variables. For this reason we decided to average data of the two years to gain clarity in the output display. The model proposed (Tab. 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* feroulyl tartaric acid and malvidin 3-*O*-glucoside) were associated to PRIN1. On PRIN2 we found variables associated to the hydroxycinnamate metabolism, namely the percentages of caffeoyl tartaric acid on one hand and of p-coumaroyl tartaric acid on the other; as expected and already discussed, these two variables were negatively correlated each other. Quercetin 3-*O*-glucuronide was negatively associated to the third principal component (PRIN3). Individuals located on the positive part of the PRIN2 axis (Fig. 3) were rich in *p*-coumaroyl tartrates (at least 70 % of total concentration), and *viceversa* 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 (Fig. 3).

Discussion

Due to a world-wide spread and to a long history of cultivation, several thousands of grape cultivars exist, that represent a wealth of metabolic diversity, partly exploited today but still very promising for the future. Characterization of this diversity is important in order to: a) provide new genotypes for quality winemaking and for health protection purposes; b) to design enological techniques adapted to specific cultivars; c) to draw hypotheses on the biosynthetic pathways underlying fruit composition; d) to provide chemotaxonomic models to be used in the study of genetic relationships and to help assessing the varietal composition of musts and, potentially, of wines.

In order to contribute to this characterization, in this study we analysed 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 polyphenolic composition of berry skins of these genotypes, involving both the coloured compounds and other phenolic classes (flavonols, HCTs) that contribute to the wine technological 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 colour intensity and hue. In the vinification process of coloured 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 whose anthocyanin profile is dominated by tri-hydroxylated 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 colour 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 colour and of 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 (*37*). In wine, HCT contents decrease during ageing with a parallel increase in oxidative browning (absorbance at 420 nm) (*38*). The intensity of browning phenomena is mainly related to *cis* and *trans* caffeoyltartaric acid content which depends on the variety (38). Consequently, the wines produced by Nascetta, Moscato bianco and Chardonnay, whose grapes contained higher concentration of trans caffeoyl tartaric 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 hypothesis find confirmation in the literature: when blends of grapes containing Chardonnay were used during the Cava sparkling wine production, they underwent browning more often than musts subjected to the same processes but without Chardonnay grapes (38). 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 (*39*).

Affecting colour, HCTs are also linked to off-odour 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-ethylguaiacol and 4-ethylcatechol) (*40*). 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 (*40)*. So, 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 it is still obscure in grape. Anthocyanin acyltransferases (AATs) have been isolated in a few plants and are part of the BAHD subfamily of acyltransferases (*41*). Reported AATs of different species act equally well on different anthocyanidin substrates (*42*). 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 coloured 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 (*42*). 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, while four of them, (Barbera, Cabernet Sauvignon, Pignola, and Teinturier elliptic 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 *Vitis vinifera* have been well characterized (*17, 43*). 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 while UFGT is expressed only after this ripening stage. This opens the possibility that flavonols are glucosylated by a specific enzyme. While anthocyanins in grape are constantly glucosylated, flavonols are also glucuronylated *(14, 15, 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. An UDP-glucuronyltransferase acting on flavonols (VvGT5) in the grape berry skin has been recently described *(44)*. Consistently with our results, expression of this gene is high already before véraison, and the recombinant enzyme shows a preference for quercetin above kaempferol *(44)*.

Biosynthesis of hydroxycinnamates in grapevine has not been detailed yet. While 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 *trans*-esterified by hydroxycinnamic acids (*45, 46*). 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* (*47*). 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 like environment, seasonal variations, etc., polyphenolic profiles (i.e. the relative amounts of each compound) are rather stable in grapes, allowing to discriminate single or groups of cultivars. In coloured cultivars, anthocyanins offer easy and largely described chemotaxonomical opportunities (*28, 48*). 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 coloured berry skin *Vitis vinifera* cultivars can be classified on the basis of the prevalence of di- or tri- hydroxylated anthocyanins and flavonols, namely cyanidin and malvidin 3-*O*-glucosides on one hand and myricetin and quercetin 3-*O*-

glucosides, on the other. Besides, we show that coloured-skin cultivars are also discriminated accordingly to the pattern of anthocyanin acylation, confirming a previous report (*48, 49*). Moreover, in this study we show that HCTs are another class of phenolic compounds, accumulating both in coloured and white cultivars, which could be effective in *Vitis vinifera* cultivar classification. The two main HCTs, namely caffeoyltartrate and *p*-coumaroyltartrate allowed variety separation upon PCA analysis. 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 to 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 non-coloured grapes, where 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 *Vitis vinifera* phenolic metabolites can improve the way of classifying cultivars. Further studies, possibly including proanthocyanidins and flavour-associated compounds, could improve classification tools and could contribute to deepen our knowledge about the biosynthetic pathways of grape secondary metabolism compounds.

Literature cited

1. Singleton, V. L.; Zaya, J.; Trousdale, E. K., Caftaric and coutaric acids in fruits of *Vitis. Phytochemistry* **1986,** 25, (9), 2127-2133.
2. Adams, D. O., Phenolics and ripening in grape berries. *American Journal of Enology and Viticulture* **2006,** 57, (3), 249-256.
3. Mané, C.; Souquet, J. M.; Olle, D.; Verries, C.; Veran, F.; Mazerolles, G.; Cheynier, V.; Fulcrand, H., Optimization of simultaneous flavanol, phenolic acid, and anthocyanin extraction from grapes using an experimental design: application to the characterization of Champagne grape varieties. *Journal of Agricultural and Food Chemistry* **2007,** 55, (18), 7224-33.
4. Cheynier, V.; Hidalgo-Arellano, L.; Souquet, J. M.; Moutounet, M., Estimation of the oxidative changes in phenolic compounds of Carignane during winemaking. *American Journal Enology Viticulture* **1997,** 48, 225-228.
5. Waterhouse, A.; Laurie, V., Oxidation of wine phenolics: a critical evaluation and hypotheses. *American Journal of Enology and Viticulture* **2006,** 57, (3), 306-313.
6. Sparvoli, F.; Martin, C.; Scienza, A.; Gavazzi, G.; Tonelli, C., Cloning and Molecular Analysis of Structural Genes Involved in Flavonoid and Stilbene Biosynthesis in Grape (*Vitis vinifera* L). *Plant Molecular Biology* **1994,** 24, (5), 743-755.
7. Boss, P. K.; Davies, C.; Robinson, S. P., Analysis of the Expression of Anthocyanin Pathway Genes in Developing *Vitis vinifera* L. cv Shiraz Grape Berries and the Implications for Pathway Regulation. *Plant Physiol* **1996,** 111, (4), 1059-1066.
8. Bogs, J.; Ebadi, A.; McDavid, D.; Robinson, S. P., Identification of the flavonoid hydroxylases from grapevine and their regulation during fruit development. *Plant Physiology* **2006,** 140, (1), 279-91.

9. Huguenеy, P.; Provenzano, S.; Verries, C.; Ferrandino, A.; Meudec, E.; Batelli, G.; Merdinoglu, D.; Cheynier, V.; Schubert, A.; Ageorges, A., A novel cation-dependent O-methyltransferase involved in anthocyanin methylation in grapevine. *Plant Physiology* **2009,** 150, (4), 2057-70.

10. Kolb, C. A.; Kaser, M. A.; Kopecky, J.; Zotz, G.; Riederer, M.; Pfundel, E. E., Effects of natural intensities of visible and ultraviolet radiation on epidermal ultraviolet screening and photosynthesis in grape leaves. *Plant Physiology* **2001,** 127, (3), 863-75.

11. Boulton, R., The copigmentation of anthocyanins and its role in the color of red wine: a critical review. *American Journal of Enology and Viticulture* **2001,** 52, 67-87.

12. Scharbert, S.; Hofmann, T., Molecular definition of black tea taste by means of quantitative studies, taste reconstitution, and omission experiments. *Journal of Agricultural and Food Chemistry* **2005,** 53, (13), 5377-84.

13. Cheynier, V.; Rigaud, J., HPLC separation and characterization of flavonols in the skins of *Vitis vinifera* var. Cinsaut. *American Journal of Enology and Viticulture* **1986,** 37, (4), 248-252.

14. Mattivi, F.; Guzzon, R.; Vrhovsek, U.; Stefanini, M.; Velasco, R., Metabolite profiling of grape: flavonols and anthocyanins. *Journal of Agricultural and Food Chemistry* **2006,** 54, (20), 7692-702.

15. Castillo-Muñoz, N; Gómez-Alonso, S.; García_Romero, E; Hermosín-Gutiérrez, I., Flavonol profiles of *Vitis vinifera* Red Grapes and Their Single-Cultivar Wines. *Journal of Agricultural and Food Chemistry* **2007,** 55, 992-1002. 16. Castillo-Muñoz, N; Gómez-Alonso, S.; García_Romero, E; Hermosín-Gutiérrez, I., Flavonol profile of *Vitis vinifera* white grape cultivars. *Journal of Food Composition and Analysis* **2010,** 23, 699-705.

17. Downey, M. O.; Harvey, J. S.; Robinson, S. P., Synthesis of flavonols and expression of flavonol synthase genes in the developing grape berries of Shiraz and Chardonnay. *Australian Journal of Grape and Wine Research* **2003,** 9, 110-121.

18. Ford, C. M.; Boss, P. K.; Hoj, P. B., Cloning and characterization of *Vitis vinifera* UDP-glucose:flavonoid 3-O-glucosyltransferase, a homologue of the enzyme encoded by the maize Bronze-1 locus that may primarily serve to glucosylate anthocyanidins in vivo. *J Biol Chem* **1998,** 273, (15), 9224-33.

19. Romeyer, F.; Macheix, J. J.; Goiffon, J. P.; Reminiac, C. C.; Sapis, J. C., The browning capacity of grapes. 3. Changes and importance of hydroxycinnamic acid-tartaric acid esters during development and maturation. *Journal of Agricultural and Food Chemistry* **1983,** 31, 346-349.

20. Falchi, M.; Bertelli, A.; Lo Scalzo, R.; Morassut, M.; Morelli, R.; Das, S.; Cui, J.; Das, D. K., Comparison of cardioprotective abilities between the flesh and skin of grapes. *Journal of Agricultural and Food Chemistry* **2006,** 54, (18), 6613-22.

21. Preys, S.; Mazerolles, G.; Courcoux, P.; Samson, A.; Fischer, U.; Hanafi, M.; Bertrand, D.; Cheynier, V., Relationship between polyphenolic composition and some sensory properties in red wines using multiway analyses. *Analytica Chimica Acta* **2006,** 563, 126-136.

22. Archier, P.; Coen, S,; Roggero, J.P., Phenolic contents of single variety wines. *Sciences des Aliments* **1992**, 12, (3), 453-466.

23. Wirth, J.; Morel-Salmi, C.; Souquet, J. M.; Dieval, J. B.; Aagaard, O.; Vidal, S.; Fulcrand, H.; Cheynier, V., The impact of oxygen exposure before and after bottling on the polyphenolic composition of red wines. *Food Chemistry* **2010,** 123, (1), 107-116.

24. Hoffmann, L.; Maury, S.; Martz, F.; Geoffroy, P.; Legrand, M., Purification, cloning, and properties of an acyltransferase controlling shikimate and quinate ester intermediates in phenylpropanoid metabolism. *The Journal of Biological Chemistry* **2003,** 278, (1), 95-103.

25. Niggeweg, R.; Michael, A. J.; Martin, C., Engineering plants with increased levels of the antioxidant chlorogenic acid. *Nature Biotechnology* **2004,** 22, (6), 746-54.

26. Ferrandino, A.; Guidoni, S., Anthocyanins, flavonols and hydroxycinnamates: an attempt to use them to discriminate *Vitis vinifera* L. cv 'Barbera' clones. *European Food Research and Technology* **2010,** 230, (3), 417-427.

27. Torchio, F.; Cagnasso, E.; Gerbi, V.; Rolle, L., Mechanical properties, phenolic composition and extractability indices of Barbera grapes of different soluble solids contents from several growing areas. *Analytica Chimica Acta* **2010,** 660, (1-2), 183-189.

28. Pomar, F.; Novo, M.; Masa, A., Varietal differences among the anthocyanin profiles of 50 red table grape cultivars studied by high performance liquid chromatography. *Journal of Chromatography A* **2005,** 1094, (1-2), 34-41.

29. Kammerer, D.; Claus, A.; Carle, R.; Schieber, A., Polyphenol screening of pomace from red and white grape varieties (*Vitis vinifera* L.) by HPLC-DAD-MS/MS. *Journal of Agricultural and Food Chemistry* **2004,** 52, (14), 4360-4367.

30. Di Stefano, R.; Cravero, The separation of hydroxycinnamates in wine. *Sciences des Aliments* **1992,** 12, 139-144.

31. Ortega-Regules, A.; Romero-Cascales, I.; Lòpez-Roca, J. M.; E, G.-P., Anthocyanin fingerprint of grapes: environmental and genetic variations. *Journal of the Science of Food and Agriculture* **2006,** 86, (10), 1460-1467.

32 de Beer, D.; Joubert, E.; Marais, J.; van Schalkwyk, D.; Manley, M., Climatic region and vine structure: effect on Pinotage wine phenolic composition, total antioxydant capacity and colour. *South Africa Journal of Enology and Viticulture* **2006,** 27, (2), 151-166.

33. Ledda, S.; Sanna; Garcia-Romero, E.; Manca, G.; Franco, M.; Porcu, A., Variability in flavonol content of grapes cultivated in two mediterranean islands (Sardinia and Corsica). *Journal of Food Composition and Analysis* **2010,** 23, (6), 580-585.

34. González-Neves, G.; Gil, G.; Barreiro, L., Influence of grape variety on the extraction of anthocyanins during the fermentation on skins. *European Food Research and Technology* **2008,** 226, 1349-1355.

35. Mazza, G.; Fukumoto, L.; Delaquis, P.; Girard, B.; Ewert, B., Anthocyanins, phenolics, and color of Cabernet Franc, Merlot, and Pinot Noir wines from British Columbia. *J Agric Food Chem* **1999,** 47, (10), 4009-17.

36. Downey, M. O.; Harvey, J. S.; Robinson, S. P., The effect of bunch shading on berry development and flavonoid accumulation in Shiraz grapes. *Australian Journal of Grape and Wine Research* **2004,** 10, (1), 55-73.

37. Singleton, V. L.; Salgues, M.; Zaya, J.; Trousdale, E. K., Caftaric acid disappearance and conversion to products of enzymic oxidation in grape must and wine. *American Journal Enology Viticulture* **1985,** 36, (1), 50-56.

38. Ibern-Gómez, M.; Andrés-Lacueva, R.; Lamuela-Raventó, S.; Buxaderas, S.; Singleton, V. L.; de la Torre-Boronat, M., Browning of cava (sparkling wine) during aging in contact with lees due to the phenolic composition. *American Journal Enology Viticulture* **2000,** 51, (1), 29-36.

39. Corona, A., Wine-making with protection of must against oxidation in a warm semi-arid terroir. *South African Journal of Enology and Viticulture* **2010,** 31, 58-63.

40. Gerbaux, V.; Vincent, B.; Bertrand, A., Influence of maceration temperature and enzymes on the content of volatile phenols in Pinot noir wines. *American Journal of Enology and Viticulture* **2002,** 53, (3), 131-137.

41. D'Auria, J. C., Acyltransferases in plants: a good time to be BAHD. *Curr Opin Plant Biol* **2006,** 9, (3), 331-40.

42. Nakayama, T.; Suzuki, H.; Nishino, T., Anthocyanin acyltransferases: specificities, mechanism, phylogenetics, and applications. *Journal of Molecular Catalysis B-Enzymatic* **2003,** 23, (2-6), 117-132.

43. Davies, C.; Robinson, S. P., Sugar accumulation in grape berries. Cloning of two putative vacuolar invertase cDNAs and their expression in grapevine tissues. *Plant Physiol* **1996,** 111, (1), 275-283.

44. Ono, E.; Homma, Yu; Horikawa, M.; Kunikane-Doi, S.; Imai, H.; Takahashi, S.; Kawai, Y.; Ishiguro, M.; Fukui, Y.; Nakayama, T., Functional differentiation of the glycosyltransferases that contribute to the chemical diversity of bioactive flavonol glycosides in grapevines (*Vitis vinifera*). Plant Cell **2010**, 22, 2856-2871.

45. Villegas, R. J.; Kojima, M., Purification and characterization of hydroxycinnamoyl D-glucose. Quinate hydroxycinnamoyl transferase in the root of sweet potato, Ipomoea batatas Lam. *The Journal of Biological Chemistry* **1986,** 261, (19), 8729-33.

46. Strack, D., Development of 1-O-sinapoyl-β-D-glucose: L-malate sinapoyltransferase activity in cotyledons of red radish (*Raphanus sativus* L. var. sativus). *Planta* **1982,** 155, 31-36.

47. Hohlfeld, M.; Veit, M.; Strack, D., Hydroxycinnamoyltransferases involved in the accumulation of caffeic acid esters in gametophytes and sporophytes of *Equisetum arvense*. *Plant Physiol* **1996,** 111, (4), 1153-1159.

48. Núñez, V.; Monagas, M.; Gomez-Cordovés, M.; Bartolomé, B., *Vitis vinifera* L. cv. Graciano grapes characterized by its anthocyanin profile. *Postharvest Biology and Technology* **2004**, 31, (1), 69-79.

49. González-Neves, G.; Gómez-Cordovés, C.; Barreiro L., Anthocyanic composition of Tannat, Cabernet Sauvignon and Merlot young red wines from Uruguay. *Journal of Wine Research* **2001,** 12, (2), 125-133.

Captions

Table 1 - Grape genotypes profiled in this study, their geographic distribution and main characteristics and dates of harvest in the two years of study. In the first column, the abbreviations used in the Principal Component Analysis output is shown in parentheses.

Table 2 - Total anthocyanin concentrations (Total Anth) and anthocyanin profiles (%) of the skins of some coloured cultivars in two successive years. 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 $P \leq 0.05$ = *; $P \leq 0.01$ = ** ; $P \leq 0.0001$ = ***.
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 non acylated tri-hydroxylated anthocyanins and total free di- = sum of the percentages of non acylated di-hydroxylated anthocyanins.

Table 3 - Total flavonol concentrations (Total Flav) and flavonol profiles (%) of the skin of the coloured grape cultivars in two successive years.
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 $P \leq 0.05$ = *; $P \leq 0.01$ = ** ; $P \leq 0.0001$ = ***.

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 glycosydes; total Ks = sum of kaempferol glycosydes; Myr/Qs = ratio myricetin 3-O-glucoside/sum of quercetin glycosydes.

Table 4 - Total flavonol concentrations (Total Flav) and flavonol profiles (%) of the skins of the white grape cultivars in two successive years.

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 $P \leq 0.05$ = *; $P \leq 0.01$ = ** ; $P \leq 0.0001$ = ***; ns = non significant.

Q 3*O*gl = quercetin 3-*O*-glucuronide; Q 3*O*G = quercetin 3-*O*-glucoside; K 3*O*gl = kaempferol 3-*O*-glucuronide; K 3*O*G = kaempferol 3-*O*-glucoside; sum of Qs = sum of quercetin glycosydes; sum of Ks = sum of kaempferol glycosydes.

Table 5 - Total hydroxycinnamates (HCTs) and HCT profiles (%) of the coloured grape cultivars in two successive years.

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 $P \leq 0.05$ = *; $P \leq 0.01$ = ** ; $P \leq 0.0001$ = ***; ns= non significant.

trans CT = trans caffeoyltartaric acid; cis *p*-coumT = cis *p*-coumaroyltartaric acid; trans *p*-coumT = trans *p*-coumaroyltartaric acid; trans fT = trans ferouyltartaric acid; *p*-coum/CT = ratio *p*-coumaroyltartaric acid (*cis* + *trans*) / caffeoyltartaric acid.

Table 6 - Total hydroxycinnamates (HCTs) and HCT profiles (%) of the white grape cultivars in two successive years.

For each variety, means followed by different letters are significantly different for P ≤ 0.05. Significance of year, cultivar and interaction year*cultivar effects was tested for *P* ≤· 0.05 = *; P ≤· 0.01 = ** ; P ≤· 0.0001 = ***; ns= non significant .

trans CT = trans caffeoyltartaric acid; cis *p*-coumT = cis *p*-coumaroyltartaric acid; trans *p*-coumT = trans *p*-coumaroyltartaric acid; trans fT = trans ferouyltartaric acid; *p*-coum/CT = ratio *p*-coumaroyltartaric acid (*cis* + *trans*) / caffeoyltartaric acid.

Table 7 - Eigenvectors of the examined variables on the three principal components (PRIN1, PRIN2 and PRIN3). Eigenvalues of the three PRINs and their contribution to total variance. In bold letters the variables associated to the appropriate PRIN.

Figure 1 – Bi-dimensional 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 grey. 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 – Bi-dimensional 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 grey. 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 ferouyltartaric acid, V5 = HCT total concentration.

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

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	distribution[1]	notes	Harvest date	
			2006	2007
Alicante Bouschet (ab)	I	The well know red flesh grape variety bred by H. Bouschet in 1865 crossing Grenache (Alicante) and Petit Bouschet	28/09	12/09
Arneis	L	Reputed speciality 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 of extinction, giving beautiful coral colored grapes for table use	19/09	05/09
Barbera (brb)	I	The 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 south-eastern 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	I		05/09	21/08
Chasselas blanc	I		05/09	05/09
Cortese	L	A major white variety in Piedmont	12/09	28/08
Croatina (cro)	It	A quite important wine cultivar, mainly grown in Piedmont, Lombardy and Emilia	12/09	12/09
Dolcetto (dlc)	L	One of the most planted red in Piedmont, giving varietal colored wines of medium body	12/09	05/09
Freisa (fre)	L	Local variety from Piedmont, grown at a minor extent all over the region	12/09	28/08
Gambarossa (gro)	L	A cultivar from a restricted area nearby Asti, giving spicy, medium bodied wines	19/09	05/09
Grignolino (gri)	L	Well-known variety from Piedmont, producing wines of light colour and a dry, tannic palate	19/09	12/09
Grisa rossa (gr)	L*	Synonym 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	L	Muscat-flavoured 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	A cultivar from the Alps, nearly extinct, has a remarkable oenological 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	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	The most reputed variety of the region, giving top quality wines among which 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	An 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 nearby 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 flavour, used as Teinturier in older vineyards	05/09	21/08

[1] L, local; L*, local with synonyms in other regions; It, Italian; I, international

Table 1

		Total Anth (mg kg^{-1})	Df	Cy	Pt	Pn	Mv	acetyl	p-coum	caff	tot free tri-	total free di-	tot 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
coloured-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
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 (elliptic 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	**	**	***	***

Table 2

		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*	
coloured-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
	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
		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 elliptic 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		***		***		***		***		***		***		***		***		***		***	

Table 3

		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		*	***	***	***	***	***	***	***

Table 4

		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		***	***	***	***	***	***

Table 6

	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 ferouyliltartaric 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
Eingenvalues	5..56	2.56	1.94
Total variance	0.37	0.18	0.13

Table 7

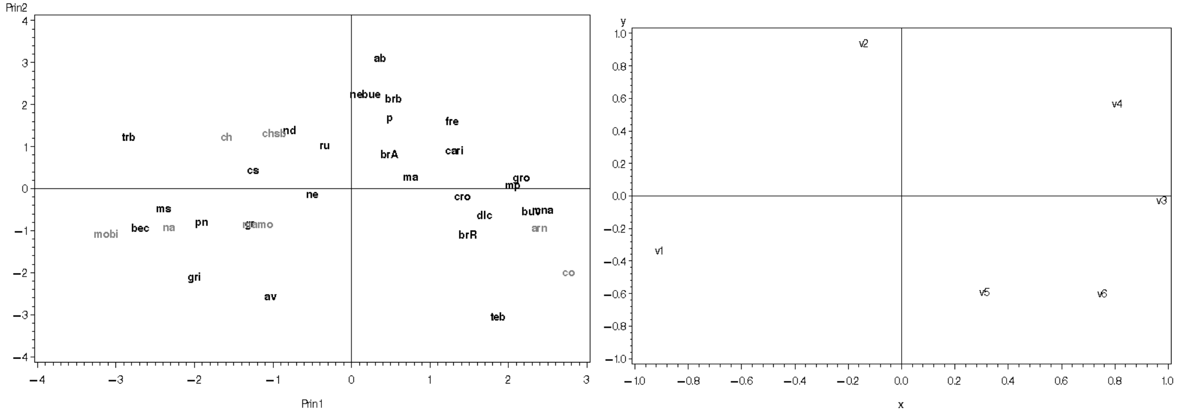

Figure 1

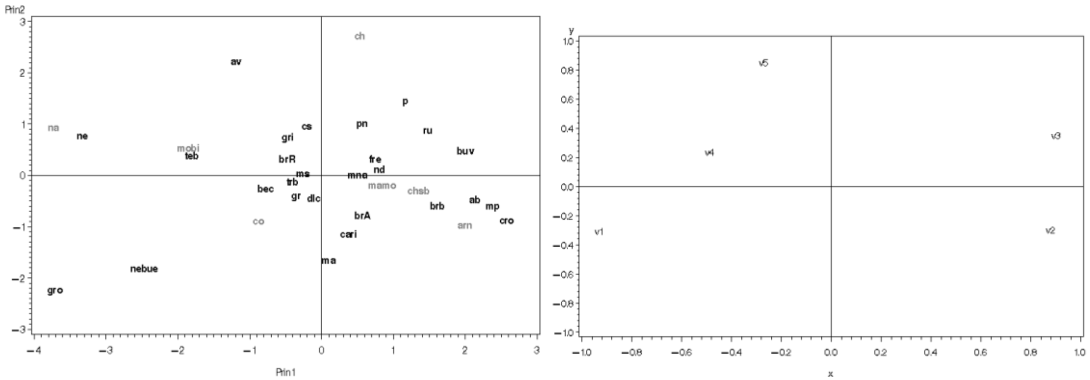

Figure 2

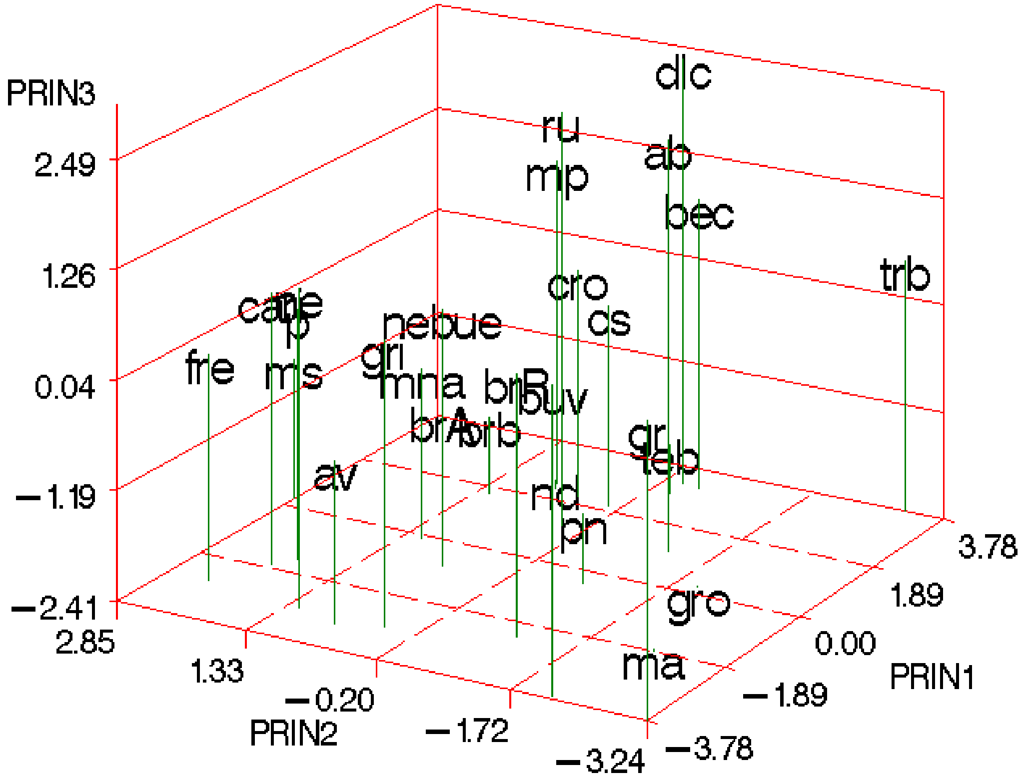
PRIN3
2.49
1.26
0.04
−1.19
−2.41
2.85
1.33
−0.20
PRIN2
−1.72
−3.24
−3.78
−1.89
0.00
1.89
3.78
PRIN1
dlc
ru
ab
mp
bec
trb
cro
cs
gri
mna
fre
ms
av
buv
nd
pn
gro
ma
teb

Figure 3

		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
Barbarossa (uva reina)	2007	36.3 b	42.0 b	13.5 a	43.8 a	0.7	1.4 a
Grec rouge	2006	38.2	47.1	13.7	39.1	0.0 b	1.1
Grisa roussa (Grec rouge)	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*
coloured-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
Barbera	2007	78.9	38.5 b	7.2	53.5 a	0.8 b	1.6 a
Becuet	2006	36.7 b	49.6 a	6.9	40.8 b	2.8	1.0 b
Becuet	2007	47.7 a	44.4 b	7.0	46.0 a	2.6	1.2 a
Brachetto (from Acqui)	2006	29.6 b	38.6 b	12.8 a	48.0 a	0.6 a	1.6 a
Brachetto (Acqui)	2007	51.9 a	44.8 a	9.7 b	45.2 b	0.3 b	1.2 b
Brachetto (from Roero)	2006	70.8	48.3 a	7.9	43.0 b	0.8 b	1.0 a
Brachetto (Roero)	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
Cabernet sauvignon	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
Cari (Pelaverga)	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
Croatina 43	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
Dolcetto	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
Freisa	2007	26.5 a	20.7	11.7	67.6 a	0.0	3.8
Gamba rossa	2006	115.1	78.4 a	2.2 b	18.6 b	0.7	0.3 b
Gamba di pernice	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
Grignolino	2007	40.9 b	38.9 a	8.6	51.7	0.8 a	1.6
Grec rouge	2006	38.2	47.1	13.7	39.1	0.0 b	1.1
Grisa roussa (Grec rouge)	2007	29.1	56.5	13.0	29.7	0.7 a	0.8
Malvasia di Schierano	2006	74.2 a	24.4 b	10.3	65.3 a	0.0	3.1 a
Malvasia di Schierano	2007	55.6 b	26.4 a	8.8	64.1 b	0.7	2.8 b
Montanera (di Perosa)	2006	58.7	28.2	8.9	60.0	2.8 b	2.4
Montanera (di Perosa)	2007	54.4	26.1	8.4	60.5	4.9 a	2.6
Muskat Hambourg	2006	16.6 b	38.5	5.1	20.0 b	1.3	0.3
Moscato d'Amburgo	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
Moscato nero d'Acqui (Malaga)	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
Nebbiolo	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
Nebue	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
Neretto duro (Balau)	2007	43.8	41.8 b	6.9 a	49.8 a	1.6 b	1.4 a
Pignolo spano (Pignola)	2006	41.0 a	28.5 b	11.0	59.9 b	0.0	2.5 a
Pignolo spano (Pignola)	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
Pinot noir	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
Ruchè	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
Neirano (Alicante Bouschet)	2007	102.3	44.3	4.2	49.2	2.2	1.2
Jacquez	2006	107.1	73.2	4.1	24.8	0.7	0.4
Uva fogarina (Jacquez)	2007	93.0	68.8	4.6	25.9	0.7	0.4
Teinturié (elliptic berry)	2006	65.0	51.9 b	3.6 b	40.7	3.7	0.8 a
Teinturié (acino ellittico, Lacrima cristi)	2007	68.9	56.3 a	4.8 a	36.4 b	2.5	0.7 b
Teinturié (round berry)	2006	63.6	64.1 a	2.4	30.0 a	5.2 b	0.5 b

Teinturié (acino rotondo)	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		***		***		***		**		***		***	

800 Table 5