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# Anthocyanin and aroma profiling of the 'Albarossa' grapevine crossbreed (*Vitis vinifera* L.) and its parent varieties 'Barbera' and 'Nebbiolo di Dronero'

P. Cook Papini[1)], G. Mazza[2)], M. Gatti[1)] and L. Bavaresco[1)]

[1)] Istituto di Frutti-Viticoltura, Università Cattolica del Sacro Cuore, Piacenza, Italy
[2)] Enosis Meraviglia, Fubine, Italy

## Summary

***V. vinifera* L. 'Barbera', 'Nebbiolo di Dronero' and the crossbreed 'Albarossa', grown in Piedmont region, Italy, were characterized by the analysis of grape anthocyanins, using High Performance Liquid Chromatography (HPLC), and aromatic compounds using Gas Chromatography coupled with Mass Spectrometry (GC-MS). Five monomeric non-acylated anthocyanins, delphinidin-3-monoglucoside, cyanidin-3-monoglucoside, petunidin-3-monoglucoside, peonidin-3-monoglucoside, malvidin-3-monoglucoside, and the pool of acetic acid acylated anthocyanins and coumarate/caffeoate anthocyanins were detected, as well as the concentration of terpenes, norisoprenoids, alcohols and benzene compounds. Ratios between the different anthocyanin forms were used for varietal profiling, as well as ratios and concentrations of single or pooled aromatic compounds. 'Albarossa' had intermediate levels, between 'Barbera' and 'Nebbiolo di Dronero', of certain anthocyanins and aromas, due to the genetic relationships.**

K e y w o r d s : grapevine, 'Albarossa', 'Barbera', 'Nebbiolo di Dronero', anthocyanins, aromas, terpenes, norisoprenoids, benzenoids.

## Introduction

The description and characterization of wine grape varieties (*Vitis vinifera* L.) has an important role in viticulture and oenology. Various methods are implicated including the analysis of secondary metabolites such as anthocyanins (for red grapes), aromatic compounds and tannins (Wenzel *et al.* 1987, Bayonove *et al.* 1998, Mattivi *et al.* 2009). Even though the grape concentrations of these components can be affected by environmental conditions and vineyard management (Jackson and Lombard 1993), the ratios among the single components of anthocyanins and aromas are controlled by the genotype. Extensive data is available in literature about anthocyanins which differ across red varieties when considering the ratios among monomers and the ratios between non-acylated and acylated molecules (Scienza *et al.* 1986, Wenzel *et al.* 1987, Mazza and Minata 1993, Calò *et al.* 1994, Boss *et al.* 1996, Costacurta *et al.* 2001, Mateus *et al*. 2002, Borsa *et al.* 2005, Pomar *et al.* 2005, Mattivi *et al.* 2006).

Grape and wine aromas have been the subject of widespread study for chemical characterization objectives and as a discrimination tool across varieties (Bayonove *et al.* 1998, Oliveira *et al.* 2000, Fernández-González *et al.* 2003, Swiegers *et al.* 2005). Aromatic compounds involved in varietal characterization are mainly monoterpenes, C13-norisoprenoids, methoxypyrazines, sulfur compounds, benzene compounds and C6 alcohols (Ribéreau-Gayon *et al.* 2000, Oliveira *et al.* 2006).

Terpenes can be found as free-forms that present characteristic muscat aromas and more often as glycosylated odourless precursors (Mazza *et al.* 2003, Conde *et al.* 2007), and ratios among monomers are related to the grape cultivar (Cordonnier and Bayonove 1974, Günata *et al.* 1985, Mateo and Jiménez 2000).

Norisoprenoids are C9-C13 fragments resulting from the degradation of carotenoids of 40-carbon atoms and these can also be found as bound precursors (Ranzungles *et al.* 1993). The C13-norisoprenoids are the most interesting compounds due to their particular aromatic properties and two examples found in grapes are, β-damascenone which gives flower, tropical fruit and stewed apple aromas, and β-ionone which gives violet aromas (Schreier *et al.* 1976, Conde *et al.* 2007). C13-norisoprenoids are deemed to be the aromatic compounds which contribute most to the aromas found in wines which are made from neutral grape varieties (Cabrita *et al.* 2006). As of yet, norisoprenoid grape composition has not been used for varietal characterization.

C6 alcohols, as for example 1-hexanol, *trans*- and *cis*-hexenol are found as odorants in wines, linked with green aromas (Herraiz *et al.* 1990) and they are related to the grape variety and to the degree of grape ripening (Cordonnier and Bayonove 1981).

Volatile benzene compounds, such as benzoic and salicylic acids, are important precursors for the formation and synthesis of various volatile components giving rise to floral and fruity flavours (Marasco and Schmidt-Dannert 2007).

HPLC has become the method for the separation and analysis of phenolic compounds, including anthocyanins from grape skins (Wulf and Nagel 1978, Hong and Wrolstad 1990). In addition, grape and wine phenolics show characteristic absorbencies in the Visible UV spectrum and hence can be detected in an efficient manner in HPLC by a photodiode array detector. Anthocyanins are red-colored and can be detected due to their absorbance at around 520 nm (Gomez-Alonso *et al.* 2007).

Correspondence to: Prof. Dr. L. Bavaresco, Istituto di Frutti-Viticoltura, Università Cattolica del Sacro Cuore, 29100 Piacenza, Italy. Fax: +39 0523 599268. E-mail: luigi.bavaresco@unicatt.it

Due to significant advances in the field, the selective retention of aromatic compounds on solid-phase absorbent columns can be used to separate them (Mateo and Jiménez 2000). Williams *et al.* (1982), applied glass column chromatography which contained C18 reversed phase adsorbent to extract glycosides, since hydrophilic compounds can be eluted with water, free-terpenes with dichloromethane and finally glycosides with methanol. This method has been greatly employed and improved in recent years and has proved useful for the isolation of glycosides (Mazza *et al.* 2003, Mateo and Jiménez 2000, Di Stefano *et al.* 2000).

After glycoside precursors isolation, the evaluation of the volatile aromatic compounds which can be released from these precursors, is completed using Gas Chromatography (GC) coupled with Mass Spectrometry (GC-MS). The use of GC is limited to the separation of volatile derivatives of the glycosides and the coupling of GC with MS, allows for the separation and identification of glycosides given characteristic fragmentation patterns. The fragmentation patterns can allow for the detection of individual glycosides and their aglycones (Mateo and Jiménez 2000).

The objective of this study was to characterize the Albarossa grapevine crossbreed and the parents of the cross, 'Barbera' and 'Nebbiolo di Dronero' grape varieties, through the analysis of grape anthocyanins and certain aromatic compounds.

## Material and Methods

Plant material: Grapes of *V. vinifera* L. 'Albarossa' and 'Barbera' were hand harvested on September 20, 2007, in the experimental vineyard of the Enosis Meraviglia research center, located in Fubine (Piedmont, Italy, 44°57' N, 8°25' E, 192 m asl), while 'Nebbiolo di Dronero' was hand harvested on September 11, 2007 in a commercial vineyard located in Costigliole Saluzzo (Piedmont, Italy, 44°34' N, 7°29' E, 460 m asl). The meteorological conditions of the two vineyards, during 2007, were similar in terms of temperature, relative humidity and rainfall. The heat summation (degree days, from April till October) was 1876 °C for Fubine and 1775 °C for Costigliole Saluzzo, while the average temperatures of the ripening time (August and September) were 21.2 °C and 17.5 °C for Fubine and 21.2 °C and 17.1 °C for Costigliole Saluzzo. The average relative humidity and the number of rainy days of the ripening time (August and September) were 66.1 %, 67.9 %, and 14, 15 for Fubine and Costigliole Saluzzo respectively. Grapes were stored at -20 °C.

Anthocyanin extraction: Ten berries per cluster were randomly and individually selected from 50 grape bunches for each variety. Each berry was separated from the pedicel and the uniformity verified. The grape skins were separated from the pulp and placed in a flask containing 25 mL of tartaric buffer of pH = 3.2 (5 g of tartaric acid, 22 mL of NaOH 1 N, 500 mL of double distilled water, 2 g of sodium metabisulfite, 120 mL of 95 % ethanol). Skins were left in the buffer for at least 4 h at room temperature before homogenization and consequently centrifugation. The supernatant was collected in a volumetric flask and the residue was washed over again with buffer added to the volumetric flask and then concentrated using a 300 mg C18 cartridge (300 mg C18 Sep-Pak Waters cod. WAT051910) which was activated with 2 mL methanol and 2 mL 0.01 M $H_2SO_4$. The skin extract in the tartaric buffer of pH = 3.2, was passed across the column with 2 mL 0.1 N $H_2SO_4$. The cartridge was washed with 2 mL 0.01 N $H_2SO_4$ and anthocyanins were eluted with 3 mL methanol into a distillation bulb. The liquid solvent was evaporated under vacuum at 30 ºC. The fraction was then recovered with 1 mL $H_3PO_4$ $10^{-3}$ M and $CH_3OH$ (60:40). After filtration through a 0.20 µm membrane the extract was ready for analysis by HPLC.

HPLC conditions: A Perkin Elmer (Waltham, MA) Mod. Series 200 high-performance liquid chromatograph equipped with a PE LC-250B Pump with Series 200 Autosampler and a Perkin Elmer Series 2000 photodiode array detector (DAD) was used. The HPLC column used was a LiChroCART 250-4 Lichrospher 100 RP-18, 5 µm particle size, 250 mm x 4 mm I.D. (Merck) and this was protected with LiChroCART 4-4 Lichrospher 100 RP-18, 5 µm particle size precolumn (Merck). A volume of 150 µL of the extract solutions was injected through the RP-18 column for analytical HPLC. The flow rate was established to 0.6 mL·min$^{-1}$ and the mobile phase consisted of a 10 % formic acid as solvent A and 10 % formic acid/50 % methanol as solvent B. The gradient profile was 72 % of solution A at 5 min, 55 % of solution A at 25 min, 30 % of solution A at 35 min, 10 % of solution A at 40 min, 1 % of solution A at 45 min, 1 % of solution A at 57 min, 72 % of solution A at 60 min. The mobile phase was returned to the initial conditions in 3 min. Data were recorded on an externally connected computer with the TotalChrom Client Server Software version 6.2.0 from Perkin Elmer. The chromatograms were collected at 520 nm wavelength and the photodiode array spectra were recorded between 5 nm and 695 nm. Delphinidin-3-monoglucoside, cyanidin-3-monoglucoside, petunidin-3-monoglucoside, peonidin-3-monoglucoside and malvidin-3-monoglucoside and their corresponding acylated forms were identified and quantified. The HPLC results were compared to a typical HPLC for anthocyanin identification in grape berry skins. The characteristic retention times were observed and consequent identification of the individual anthocyanins was completed. In addition to the 5 non-acylated anthocyanins, acetic acid acylated anthocyanins and the coumarate and caffeoate anthocyanins were also identified and quantified. By the integration of the chromatogram, it was possible to determine the areas of the peaks, and therefore to calculate the corresponding ratios of the various anthocyanins.

Aroma extraction: Berries were randomly and individually selected from the grape bunches. Each berry was separated from the pedicel, integrity was verified and approximately 80 g were weighed. The grape berry seeds were removed and the skins and pulp were homogenized. The mixture was centrifuged and the supernatant was collected in a volumetric flask. The solid phase was then washed and re-suspended in 40 mL of buffer for 30 min. It was then centrifuged, washes were repeated 3 times and subsequently added to the volumetric flask. Once complet-

ed 200 µL of pectolytic enzyme was added to the volumetric flask and left for at least 1 h after which the extract was filtered. The isolation of varietal aroma compounds was completed following similar methods to those used by other authors (Williams *et al.* 1982, Di Stefano *et al.* 2000, Cabrita *et al.* 2006), although with some modifications, as follows. The filtered extract was passed through a 5 g Isolute C18 cartridge (International Sorbent Technology, UK) which was activated prior with first 25 mL methanol and 35 mL double distilled water. Once the extract passed through the cartridge, it was washed with 30 mL water. The cartridge was eluted with 40 mL dichloromethane, this eluted fraction was eliminated because little or no free volatile compounds are present in these neutral grapes. The compounds of interest (glycosylated varietal aromas) were eluted with 30 mL methanol and then evaporated under vacuum at 40 ºC; the residue was then recovered with 5 mL citrate-phosphate buffer at pH 5. Then 0.2 mL of a high glycosidic activity enzyme was added and the solution was incubated at 40 ºC overnight. An aliquot (0.4 mL) of internal standard (2-Ottanol) was added after incubation and this mixture was then passed through a 1 g Isolute C18 cartridge (International Sorbent Technology, UK) which was activated prior with 15 mL methanol and 30 mL double distilled water. The column was washed with 20 mL water, after which an elution with dichloromethane was completed to recuperate the aglycones produced by enzymatic hydrolysis of the glycosylated forms. The dichloromethane extract was dried by the addition of anhydrous sodium sulfate and was concentrated by distillation. Once concentrated, this fraction was ready for GC-MS analysis.

GC-MS conditions: An Agilent 6890N Network GC system linked on line with a Mass Selective Detector MSD 5973 Network (Agilent) equipped with a SupelCoWax 10 (SupelCo) 30 m x 0.25 mm i.d and 0.25 µm df column was utilized. The oven was programmed starting from 45 ºC, kept constant for 2 min and linearly increasing up to 60 ºC at the rate of 30 ºC/min. This was held for another 2 min, after which it was increased at 2 ºC/min up to 160 ºC, followed by an increase to 230 ºC at 5 ºC·min$^{-1}$ for 25 min. The following experimental conditions were applied: injector temperature 250 ºC; injection mode: splitless for 2 min; acquisition mass range 28-300 u.m.a; ionization energy 70 eV; carrier gas helium at a flow of 1 mL/min.

Compounds identification and quantification: The internal standard 2-Octanol was applied for all analyses. Compounds were then identified by comparing the retention times and MS spectra with standards, data from previously completed trials, from experience and furthermore by comparing with results of retention times and MS spectra from available literature. Semi-quantitative data was obtained by comparing the ratio peak height of the individual compounds of interest with that of the internal standard peak height.

## Results and Discussion

HPLC separation of anthocyanins: Identical peak patterns were obtained for all three grape varieties, 'Albarossa', 'Barbera' and 'Nebbiolo di Dronero'. The peak retention times were similar and an average retention time was used for identification. Peak identification was completed by comparing with previous HPLC chromatograms. Furthermore using the literature available it was possible to identify the peaks as they follow the same general pattern (Fig. 1).

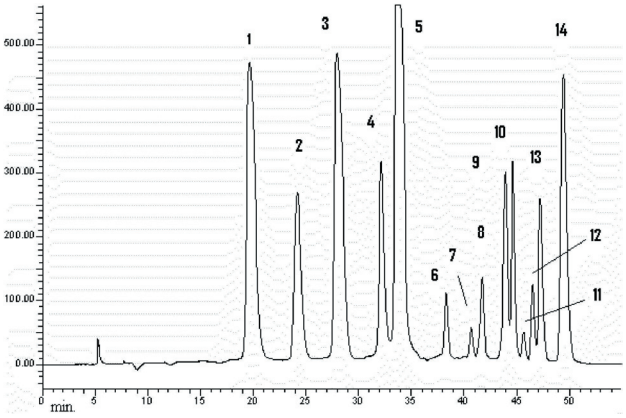


Fig.1: Chromatographic pattern of 'Albarossa' grape skin anthocyanins. **1**: delphinidin-3-monoglucoside; **2**: cyanidin-3-monoglucoside; **3**: petunidin-3-monoglucoside; **4**: peonidin-3-monoglucoside; **5**: malvidin-3-monoglucoside; **6**: delphinidin-3-monoglucoside acetate; **7**: cyanidin-3-monoglucoside acetate; **8**: petunidin-3-acetylglucoside; **9**: peonidin-3-monoglucoside acetate + malvidin-3-monoglucoside acetate; **10**: delphinidin-3-monoglucoside coumarate; **11**: malvidin-3-monoglucoside caffeoate; **12**: cyanidin-3-monoglucoside coumarate; **13**: petunidin-3-monoglucoside coumarate; **14**: peonidin-3-monoglucoside coumarate + malvidin-3-monoglucoside coumarate.

The 3-monoglucosides eluted first, beginning with delphinidin, followed by cyanidin, petunidin, peonidin and malvidin. This same order was noted for the acetic-acid-acylated anthocyanins which are subsequently eluted. Finally, also in the same anthocyanidin order, the coumarates and caffeoates were eluted.

Anthocyanin characterization of grape varieties: The chromatograms were used to calculate the relative area values for each peak. Using these relative area values it was possible to obtain semi-quantitative data to make comparisons and ratios between the different anthocyanins. The relative percentages of the five grape anthocyanins (delphinidin, cyanidin, petunidin, peonidin and malvidin) were calculated for the three grape varieties, the ratios of nonacylated, acetic-acid acylated and coumarate and caffeoate acid anthocyanins were also calculated (Fig. 2). Only malvidin-caffeoate was detected, at very low levels, being 0.51 % in 'Albarossa', 0.22 % in 'Barbera' and 0.15 % in 'Nebbiolo di Dronero'.

As reported in the literature for other *Vitis vinifera* grape varieties (Mateus *et al*. 2002, Gomez-Alonso *et al.* 2003, Mannini *et al.* 2004), malvidin-3-glucoside was present in the highest amount, followed by petunidin-3-glucoside, delphinidin-3-glucoside, peonidin-3-glucoside and cyanidin-3-glucoside. 'Nebbiolo di Dronero' had a higher percentage of malvidin-3-glucoside and peonidin-3-glucoside and a lower percentage of petunidin-3-glu-

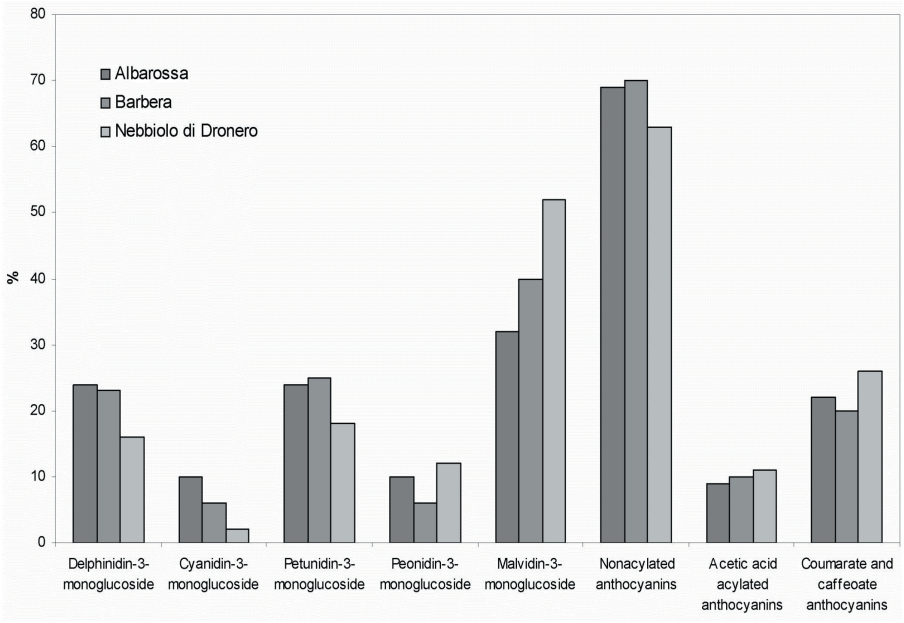


Fig 2: Percentages of monomeric nonacylated anthocyanins of 'Albarossa', 'Barbera' and 'Nebbiolo di Dronero' grapes, and percentages of nonacylated and acylated anthocyanins.

coside, delphinidin-3-glucoside and cyanidin-3-glucoside than 'Barbera'.

All the varieties had higher percentages of nonacylated anthocyanins over acetic acid acylated, coumarate and caffeoate anthocyanins. The ratios of these different anthocyanins are also important for grape variety characterization; for instance 'Pinot Noir' has no acylated anthocyanins (Lanaridis and Bena-Tzourou 1997).

It is interesting to compare the 'Albarossa' profile with those of its parents, 'Barbera' and 'Nebbiolo di Dronero'. The 'Albarossa' anthocyanin percentage pattern is closer to that of 'Barbera' than of 'Nebbiolo di Dronero' (Fig. 2), since it is characterized as having a high presence, over 80 %, of tri-substituted nonacylated anthocyanins (malvidin, petunidin, delphinidin), with respect to the di-substituted nonacylated anthocyanins (peonidin and cyanidin), as was observed for 'Barbera'.

Varietal aroma compounds: Aromatic compounds were released by enzymatic hydrolysis in order to analyze the glycosidically bound aroma precursors by GC-MS. All the identified aromatic compounds were included into three classes which are of most interest for the varietal characterization, terpenes, norisoprenoids and alcohols and benzene compounds (Tabs 1, 2, 3).

Terpenes: These compounds were shown to represent around 4-6 % of the total composition of 'Albarossa' and 'Barbera' while for 'Nebbiolo di Dronero' the percentage was around 15 % (Fig. 3). This finding is consistent with results in literature for the terpene composition of neutral grape varieties such as 'Cabernet Sauvignon', 'Pi-

Table 1

Grape terpene compounds released by enzymatic hydrolysis from glycosylated precursors yielded from solid phase extraction, analyzed by GC-MS, depending on variety

| Terpenes ($\mu g \cdot kg^{-1}$) | Albarossa | Barbera | Nebbiolo di Dronero |
|---|---|---|---|
| *trans*-furan linalool oxide | 0.9 | 7.6 | 1.3 |
| *cis*-furan linalool oxide | 1.3 | 8.7 | 0.9 |
| α-terpineol | 2.7 | n.d. | 12.7 |
| *trans*-pyran linalool oxide | 1.3 | 11.5 | 2.0 |
| *cis*-pyran linalool oxide | 1.0 | 4.8 | 0.5 |
| Nerol | 8.5 | 7.5 | 4.7 |
| Geraniol | 77.6 | 39.2 | 20.1 |
| exo-2-hydroxy-1,8-cineole | 10.2 | 2.1 | 10.0 |
| 2,6-dimethyl-3,7-octadiene-2,6-diol | 1.4 | 3.1 | 1.8 |
| *trans*-8-OH-linalool | 29.7 | 36.8 | 31.5 |
| OH-geraniol + *cis*-8-OH-linalool | 78.9 | 51.0 | 216.3 |
| p-menth-1-ene-7,8-diol | 42.5 | 6.3 | 125.8 |
| Total | 255.9 | 178.7 | 427.7 |

Table 2

Grape norisoprenoid compounds released by enzymatic hydrolysis from glycosylated precursors yielded from solid phase extraction, analyzed by GC-MS, depending on variety

| Norisoprenoids ($\mu g \cdot kg^{-1}$) | Albarossa | Barbera | Nebbiolo di Dronero |
|---|---|---|---|
| 3,4-dihydro-3-oxoactinidol I | 21.2 | 12.9 | 12.8 |
| 3,4-dihydro-3-oxoactinidol II | 41.1 | 29.3 | 33.0 |
| 3,4-dihydro-3-oxoactinidol III | 18.4 | 19.9 | 14.3 |
| 3-OH-β-damascone | 65.8 | 82.7 | 46.5 |
| Megastigma-7-en-3,9-diol | 35.5 | 35.1 | 34.4 |
| 3-oxo-α-ionol + 4-oxo-β-ionol | 361.1 | 312.9 | 226.5 |
| 3-hydroxy-7,8-dihydro-β-ionol | 20.5 | 33.5 | 34.8 |
| 3-hydroxy-7,8-dehydro-β-ionol | 44.3 | 58.6 | 34.2 |
| Grasshopperketone + vomifoliol | 529.1 | 1050.1 | 471.3 |
| Total | 1136.9 | 1636.2 | 907.8 |

Table 3

Grape alcohol and benzenoid compounds released by enzymatic hydrolysis from glycosylated precursors yielded from solid phase extraction, analyzed by GC-MS, depending on variety

| Alcohols and benzenoids ($\mu g \cdot kg^{-1}$) | Albarossa | Barbera | Nebbiolo di Dronero |
|---|---|---|---|
| Benzyl alcohol | 748.9 | 909.1 | 427.9 |
| Phenyl ethanol | 565.4 | 213.8 | 399.6 |
| Eugenol | 3.3 | 29.8 | 1.2 |
| 4-vinylguaiacol | 263.7 | 406.4 | 109.7 |
| 2,6-dimethoxyphenol | 51.4 | 42.5 | 17.2 |
| Isoeugenol | 5.6 | 10.1 | 2.4 |
| 4-vinylphenol | 459.0 | 187.1 | 128.5 |
| Vanillin | 52.7 | 24.0 | 29.8 |
| Methyl vanillate | 64.3 | 116.7 | 63.7 |
| 3,4-dimethoxyphenol | 23.4 | 10.7 | 9.9 |
| Zingerone | 26.3 | 66.1 | 16.4 |
| Butyrovanillone | 22.2 | 18.4 | 9.5 |
| Syringaldehyde | 48.7 | 8.5 | 14.5 |
| Salicylaldehyde | 26.6 | 5.4 | 5.5 |
| Dihydroconiferyl alcohol | 94.9 | 67.9 | 96.5 |
| 3,4,5-trimethoxyphenol | 186.4 | 244.9 | 156.8 |
| Total | 2642.8 | 2361.3 | 1489.3 |

not Noir' and 'Sangiovese' (Lanati *et al.* 2006). The main terpenes isolated from enzymatic hydrolysis in all varieties were OH-geraniol + *cis*-8-OH-linalool, geraniol, p-menth-1-ene-7,8-diol, *trans*-8-OH-linalool, exo-2-hydroxy-1,8-cineole and nerol (Tab. 1).

The total amount of terpenes of 'Albarossa' was in between the ones of the two parent varieties and this is the case for the majority of the single molecules, except for geraniol which was much higher than for the parents.

Furthermore, it is also noteworthy that both 'Albarossa' and 'Nebbiolo di Dronero' had higher concentrations of exo-2-hydroxy-1,8-cineole, and lower concentrations of *trans*-furan linalool oxide, *cis*-furan linalool oxide, *trans*-pyran linalool oxide and *cis*-pyran linalool oxide than 'Barbera'.

The general similarities in the relative concentrations of terpenes for each grape variety indicate the relationship between the three grape varieties.

Norisoprenoids: The total concentration ranged approximately between 30-40 % of the total glycosylated compounds (Fig. 3). The main norisoprenoids detected were, grasshopperketone + vomifoliol, 3-oxo-α-ionol +

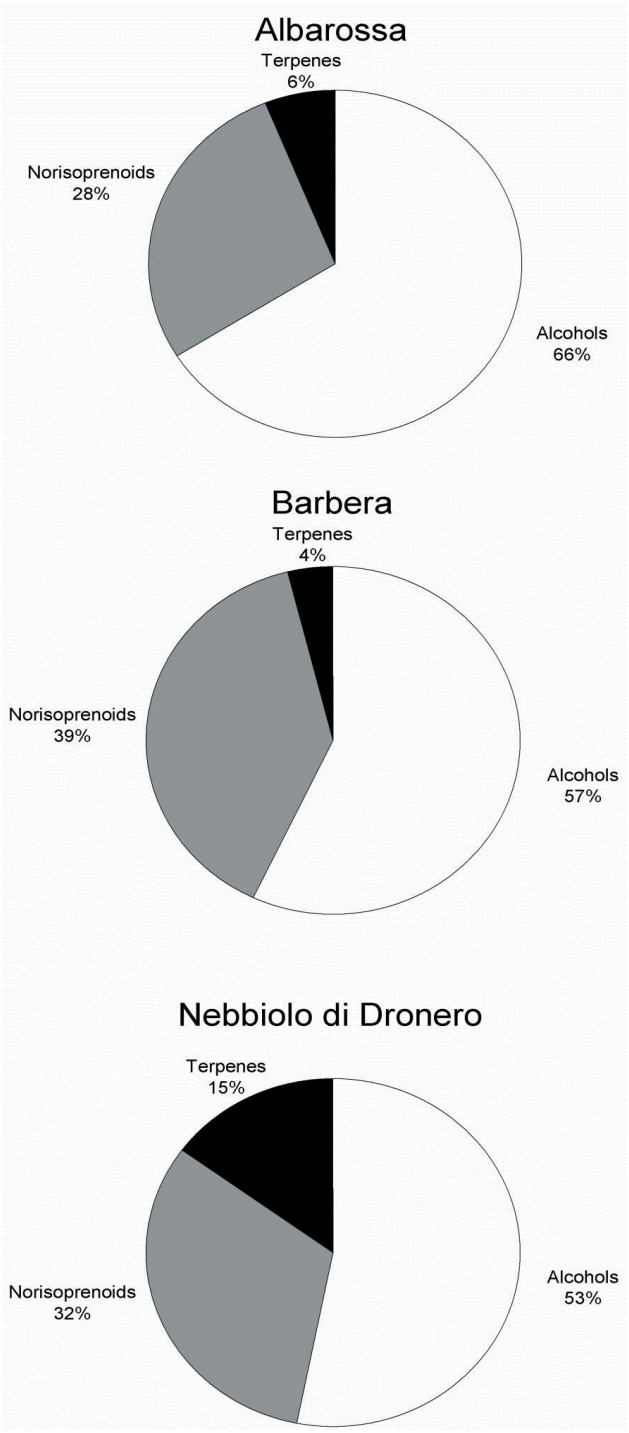


Fig 3: Percentages of terpenes, norisoprenoids and alcohols of 'Albarossa', 'Barbera' and 'Nebbiolo di Dronero' grapes.

4oxo-β-ionol and 3-OH-β-damascone (Tab. 2). The overall similarities in the norisoprenoids isolated and found in the three grape varieties demonstrates the genetic relationship between them. The relative values for all the compounds were generally similar and the most highly concentrated norisoprenoids were also similar across all the grape varieties. A common pattern was the presence of the three isomers of 3,4-dihydro-3-oxoactinidol in different relative quantities. In all the grape varieties 3,4-dihydro-3-oxoactinidol II showed the highest values followed by 3,4-dihydro-3-oxoactinidol I and 3,4-dihydro-3-oxoactinidol III. According to literature (Lanati *et al.* 2006) other varieties, such as 'Cabernet Sauvignon', show a different pattern with higher concentrations of 3,4-dihydro-3-oxoactinidol III than 3,4-dihydro-3-oxoactinidol II and 3,4-dihydro-3-oxoactinidol I. The relative quantities of these three isomers could therefore also be useful in the varietal characterization.

Grape norisoprenoids are aromatically important since they have very low sensory thresholds. These compounds are especially of interest for neutral grape varieties since they can be liberated from their bound forms during winemaking practices and consequently give free volatiles with floral and exotic fruit aromas (Lanati *et al.* 2006).

Alcohols and benzenoids: These compounds accounted for 66 % in 'Albarossa', 57 % in 'Barbera', and 53 % in 'Nebbiolo di Dronero' of the total aromas (Fig. 3). All the grape varieties showed high levels of benzyl alcohol, phenyl ethanol and several phenols, in particular, 4-vinylphenol, 4-vinylguaiacol, and 3,4,5-trimethoxyphenol (Tab. 3). High benzyl alcohol concentrations have also been observed in other grape varieties, often in cool climate regions and this compound which is released by hydrolysis of the glycosidic form during winemaking can likely be oxidized to benzylaldehyde, which can give almond aromas when found at perceptible levels (Lanati *et al.* 2006).

Ratios between compounds: According to the literature (Mazza *et al.* 2003, Cabrita *et al.* 2006, Lanati *et al.* 2006), ratios between specific aromatic molecules can be consistent within a grape variety despite changing environmental factors, such as 3-oxo-α-ionolo/3-OH-β-damascone, *trans*/*cis*-furan linalool oxide and *trans*/*cis*-pyran linalool oxide and hence can be useful in varietal characterization. In all of the grape varieties studied, the ratio of 3-oxo-α-ionolo/3-OH-β-damascone was generally greater than 1, the ratio of *trans*/*cis*-furan linalool oxide generally less than 1 and the ratio of *trans*/*cis*-pyran linalool oxide was generally greater than 1 (Tab. 1, 2).

The study allowed the varietal characterization of three genetically related grape cultivars according to their anthocyanin and aromatic profiles. Beyond this aspect, aromatic profiling can be a useful tool to predict the sensory potential of grape varieties and their corresponding wines. The work reports preliminary data, and further studies are necessary to bring some statistical significance of the results.

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## References

Bayonove, L.; Baumes, R.; Crouzet, J.; Günata, Z.; 1998: Arômes. In: C. Flanzy (Ed.): Oenologie - fondements scientifiques et technologiques, 163-235. Lavoisier Tec & Doc, Paris, France.

Borsa, D.; Carniel, D.; Asproudi, A.; Monticelli, L.; Crespan, M.; Costacurta, A.; 2005: Caratterizzazione di uve Malvasia attraverso lo studio dei metaboliti secondari. Riv. Vitic. Enol. **58**, 167-182.

Boss, P. K.; Davies, C.; Robinson, S. P.; 1996: Expression of anthocyanin biosynthesis pathway genes in red and white grapes. Plant Mol. Biol. **32**, 565-569.

Cabrita, M. J.; Costa Freitas, A. M.; Laureano, O.; Di Stefano, R.; 2006: Glycosidic aroma compounds of some Portuguese grape cultivars. J. Sc. Food Agr. **86**, 922-931.

Calò, A.; Tomasi, D.; Cravero, M. C.; Di Stefano, R.; 1994: Contributo alla caratterizzazione e classificazione varietale attraverso la determinazione degli antociani e degli HCTA della buccia di varietà a bacca rossa. Riv. Vitic. Enol. **47**, 13-25.

Conde, C.; Paulo, S.; Fontes, N.; Alberto, P.; Dias, P.; Tavares, R. M.; Sousa, M. J.; Agasse, A.; Delrot, S.; Gerós, H.; 2007: Biochemical changes throughout grape berry development and fruit and wine quality. Food **1**, 1-22.

Cordonnier, R. E.; Bayonove, C. L.; 1974: Mise en évidence dans la baie de raisin var. Muscat d'Alexandrie de monoterpènes liés revelables par une ou plusieurs enzymes du fruit. C.R. Acad. Sci. Paris **278**, 3387-3390.

Cordonnier, R.; Bayonove, C. L.; 1981: Etude de la phase prefermentaire de la vinification: extraction et formation des certains composés de l'arome: cas des terpenols, des aldéhydes et des alcohols en C6. Conn. Vigne Vin **15**, 269-286.

Costacurta, A.; Calò, A.; Crespan, M.; Milani, M.; Carraro, R.; Aggio, L.; Flamini, R.; Ajmone-Marsan, P.; 2001: Caractérisation morphologique, aromatique et moléculaire des cépages de Moscato et recherches sur leurs rapports phylogénétiques. Bull. O I V (Off. Int. Vigne Vin) **74**, 133-150.

Di Stefano, R.; Gentilizi, N.; Ammarino, I.; 2000: Studio dei profili aromatici di varietà a frutto bianco coltivate nella zona del Collio. L'Enologo **38**, 95-102.

Fernández-González, M.; Di Stefano, R.; Briones, A.; 2003: Hydrolysis and transformation of terpene glycosides from muscat must by different yeast species. Food Microbiol. **20**, 35-41.

Gomez-Alonso, S.; Garcia-Romero, E.; Hermosin-Gutierrez, I.; 2007: HPLC analysis of diverse grape and wine phenolics using direct injection and multidetection by DAD and fluorescence. J. Food Compos. Anal. **20**, 618-626.

Günata, Y. Z.; Bayonove, C. L.; Baumes, R. E.; Cordonnier, R. E.; 1985: The aroma of grapes. I. Extraction and determination of free and glycosidically bound fractions of some grape aroma components. J. Chromatogr. **331**, 83-90.

Herraiz, T.; Herraiz, M.; Regleros, G.; Martin-Alvarez, P. J.; Cabezudo, M. D.; 1990: Changes in the composition of alcohols and aldehydes of C6 chain length during the alcoholic fermentation of grape must. J. Agric. Food Chem. **38**, 969-972.

Hong, V.; Wrolstad, R. E.; 1990: Use of HPLC Separation/Photodiode Array. Detection for Characterization of Anthocyanins. J. Agric. Food Chem. **38**, 708-715.

Jackson, D. I.; Lombard, P. B.; 1993 : Environmental and management practices affecting grape composition and wine quality – A review. Am. J. Enol. Vitic. **44**, 409-430.

Lanaridis, P.; Bena-Tzourou, E.; 1997: Étude des variations des anthocyanes pendant la maturation des raisins de cinq cépage rouges, cultivé en Grèce. J. Int. Sci. Vigne Vin **31**, 205–212.

Lanati, D.; Raifer, W.; Mazza, G.; 2006: Il quadro aromatico della cultivar Cabernet Sauvignon coltivata in Alto Adige. L'Enologo **42**, 111-117.

Mannini, F.; Rolle, L.; Gerbi, V.; Zeppa, G.; Boccacci, P.; 2004: Caratterizzazione genetica e fenolica degli incroci Albarossa e Cornarea (*Vitis vinifera* L.). Vignevini **31**, 123-127.

Marasco, E. K.; Schmidt-Dannert, C.; 2007: Biosynthesis of plant natural products and characterization of plant biosynthetic pathways in recombinant microorganisms. In: R. Verpoorte *et al.* (Eds): Recombinant plant pathways in microorganisms, 1-43. Springer.

Mateo, J. J.; Jiménez, M.; 2000: Monoterpenes in grape juice and wines. J. Chromatogr. A. **881**, 557-567.

Mateus, N.; Machado, J.M.; de Freitas, V.; 2002: Development changes of anthocyanins in *Vitis vinifera* grapes grown in the Douro valley and concentration in respective wines. J. Sci. Food Agric. **82**, 1689-1696.

Mattivi, F.; Guzzon, R.; Vrhovsek, U.; Stefanini, M.; Velasco, R.; 2006: Metabolite profiling of grape: flavonols and anthocyanins. J. Agric. Food Chem. **54**, 7692-7702.

Mattivi, F.; Vrhovsek, U.; Masuero, D.; Trainotti, D.; 2009: Differences in the amount and structure of extractable skin and seed tannins amongst red grape varieties. Aust. J. Grape Wine Res. **15**, 27-35.

Mazza, G.; Miniata, E.; 1993: Grapes. In: Anthocyanins in Fruits, Vegetables, and Grain. CRC Press: Boca Raton, FL.

Mazza, G.; Cascio, P.; Barbieri, E.; 2003: Composti volatili liberi e glicoconiugati presenti nelle foglie e negli acini della *Vitis vinifera* Cv Moscato bianco. Riv. Vitic. Enol. **56**, 57-74.

Oliveira, J. M.; Maia, M. O.; Baumes, R. L.; Bayonove, C. L.; 2000: Free and bound aromatic components of Loureiro and Alvarinho grape varieties from the Vinhos Verdes region. Viti. Enol. Sci. **55**, 13-20.

Oliveira, J. M.; Faria, M.; Sá, F.; Barros, F.; Araújo, I. M.; 2006: C6-alcohols as varietal markers for assessment of wine origin. Anal. Chim. Acta **563**, 300-309.

Pomar, F.; Novo, M.; Masa, A.; 2005: Varietal differences among the anthocyanin profiles of 50 red table grape cultivars studied by high performance liquid chromatography. J. Chromatogr. A **1094**, 34-41.

Ranzungles, A.; Gunata, Z.; Pinatel, S.; Baumes, R.; Bayonove, C.; 1993: Content of terpenes, norisoprenoids and their precursors in several varieties of grapes. Sci. Aliments. **13**, 59-72.

Ribéreau-Gayon, P.; Glories, Y.; Maujean, A.; Dubourdieu, D.; 2000: Varietal Aroma. In: P. Ribéreau-Gayon; Y. Glories; A. Maujean; D. Dubourdieu (Eds): Handbook of Enology. The Chemistry of Wine Stabilization and Treatments;2nd Edition; John Wiley and Sons LTD: West Sussex, England.

Schreier, P.; Drawert, F.; Junker, A.; 1976: Identification of volatile constituents from grapes. J. Agric. Food Chem. **24**, 331-336.

Scienza, A.; Piergiovanni, L.; Visai, C.; Conca, E.; 1986: Il profilo antocianico delle uve quale mezzo tassonomico per il riconoscimento dei vitigni rossi. Vignevini **13**, 75-81.

Swiegers, J. H.; Bartowsky, E. J.; Henschke, P. A.; Pretorius, I. S.; 2005: Yeast and bacterial modulation of wine aroma and flavour. Aust. J. Grape Wine Res. **11**, 139-173.

Wenzel, W.; Dittrich, H. H.; Heimfarth, M.; 1987: Anthocyanin composition in berries of different grape varieties. Vitis **26**, 65-78.

Williams, P. J.; Strauss, C. R.; Wilson, B.; Massy-Westropp, R. A.; 1982: Use of C18 reversed-phase liquid chromatography for the isolation of monoterpene glycosides and nor-isoprenoid precursors from grape juice and wines. J. Chromatogr. **235**, 471-480.

Wulf, L. W.; Nagel, C. W.; 1978: High pressure liquid chromatographic separation of anthocyanins of *Vitis vinifera*. Am. J. Enol. Vitic. **29**, 42-49.

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