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# ANTHOCYANIN COMPOSITION OF FOUR AUTOCHTHONOUS *VITIS VINIFERA* GRAPEVINE VARIETIES FROM THE PIEDMONT

STUDIO DELLA COMPOSIZIONE ANTOCIANICA  
DI QUATTRO VITIGNI AUTOCTONI PIEMONTESE

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### ABSTRACT

The anthocyanin composition of four autochthonous grapevine varieties cultivated in the Piedmont mountains has been studied. The results showed great differences between these cultivars. The total amount of anthocyanins ranged from 74 mg/kg of *Grisa roussa* grapes to 1.024 mg/kg of *Becouet* grapes, which had the most interesting anthocyanin profile. Cyanidin-3-glucoside and peonidin-3-glucoside (72.5% of total anthocyanins) prevailed in *Avanà*; cyanidin-3-glucoside made up 94% of the total anthocyanins in *Grisa roussa*;

### RIASSUNTO

È stata analizzata la composizione antocianica di quattro vitigni autoctoni coltivati sulle montagne piemontesi. I risultati ottenuti hanno evidenziato rilevanti differenze fra le quattro cultivars. La quantità di antociani infatti è compresa fra i 74 mg/kg di uva della *Grisa roussa* ed il 1.024 mg/kg di uva del *Becouet*, ma è il profilo antocianico a fornire i dati più interessanti. La cianidina-3-glucoside e la peonidina-3-glucoside prevalgono nell'*Avanà* (72.5% delle antocianine); la cianidina-3-glucoside costituisce il 94% delle antociani-

- Key words: anthocyanin, grapevine, Piedmont -

malvidin-3-glucoside and its acyl-derivatives were highest (73%) in *Becouet* and all five anthocyanins were present in similar concentrations in *Grisa nera*.

dine nella *Grisa roussa*; la malvidina-3-glucoside ed i suoi derivati prevalgono (73%) nel *Becouet*; nella *Grisa nera* infine tutte le cinque antocianine sono presenti in concentrazione simile.

## INTRODUCTION

Phenolic compounds are responsible for some of the major sensory characteristic of wines such as colour, taste and astringency. Their concentration in wine depends on the cultivar and the wine-making conditions.

Berry skin anthocyanins change according to the nature of the substituents as well as according to the number and position of residual sugars. Chemogenetic studies suggest that a common precursor of all flavonoids is chalcone (GRISEBACH, 1982; ROGGERO *et al.*, 1986). An isomerization induced by a chalcone isomerase transforms chalcone into (-)-(2S)flavanone from which anthocyanins, flavonols and catechins are derived.

According to ROGGERO *et al.* (1986) the first hydroxylation of the flavanone gives cyanidin-3-glucoside, the most primitive pigment; cyanidin-3-glucoside is then transformed by a flavonoid 3'-hydroxylase into delphinidin-3-glucoside and by a methyltransferase into peonidin-3-glucoside and into malvidin-3-glucoside, the most stable anthocyanin pigments in grape. In the *Vitis* genus there are exclusively five anthocyanins in the glycoside form (delphinidin, cyanidin, petunidin, peonidin and malvidin). The quantity and composition of anthocyanins in the berry skin can be influenced by environmental factors and by vineyard management practices, but they vary greatly between species and varieties (BOURZEIX *et al.*, 1983; CRAVERO and DI STEFANO, 1992; CRAVERO *et al.*,

1994; DARNÉ, 1988b; DI STEFANO and MAGGIOROTTO, 1995; LEPADATU *et al.*, 1972; RIBÉREAU-GAYON, 1982; TAMBORRA and DI BENEDETTO, 1991; VAN BUREN *et al.*, 1970). In several grapevine varieties (*Cabernet franc*, *Cabernet sauvignon*, *Merlot*, *Gamay* and *Barbera*) trihydroxylated anthocyanins (delphinidin-3-glucoside, petunidin-3-glucoside and malvidin-3-glucoside) are prevalent. The wines obtained from these varieties or from others with a similar composition are characterised by a more stable colour.

In a few varieties (*Traminer*, *Gewuertztraminer*, *Muscat rosé*, *Nebbiolo*) the dihydroxylated anthocyanins, cyanidin-3-glucoside and peonidin-3-glucoside prevail.

Since the red colour of wine mainly depends on the anthocyanin composition of the grape, studies on their evolution during transformation from grape to wine are therefore of great importance. Many authors have contributed information on the anthocyanin profile in the grape skin of several vine varieties and anthocyanin evolution during grape ripening (ALBACH *et al.*, 1959; BAKKER and TIMBERLAKE, 1985; BALDI and ROMANI, 1992; CACHO *et al.*, 1992; CLIMENT and PARDO, 1997; DARNÉ, 1988a; DI STEFANO *et al.*, 1993; IEBREIRO *et al.*, 1988; HMAMOUCHE *et al.*, 1995; LANARIDIS and BENA-TZOUROU, 1997; LEE and JAWORSKI, 1989; MORASSUT and CECCHINI, 1999; MORIONDO and GENTILINI, 1992; ROGGERO *et al.*, 1986; ROSON and MOUTOUNET, 1992) and during fermentation and ageing (CHEYNIER *et al.*, 1997; DALLAS *et*

al., 1995; KOVAC, 1978; MAYEN *et al.*, 1994; NAGEL and WULF, 1979).

The purpose of this work was to study the anthocyanin composition of *Avanà*, *Becouet*, *Grisa roussa* and *Grisa nera*, old native varieties of *Vitis vinifera* growing exclusively in the Susa Valley (Turin, Italy) in vineyards located 1100 m above sea level. No studies have been conducted on these cultivars and knowledge of their anthocyanin composition is very important for improving winemaking and consequently for improving wine quality.

## MATERIALS AND METHODS

### Vines

The grapes were obtained from the Susa Valley, the most important valley in the Turin province for grape production (Piedmont, Northwest Italy). In this valley, as in Vallese in Switzerland, there are vineyards over 1,000 m above sea level. *Avanà* and *Becouet* have black-violet berries, while *Grisa nera* have blue-black berries and *Grisa roussa* pink-red berries. *Grisa roussa* is not a mutation of *Grisa nera* from which it differs greatly. The only authorised cultivar grown among these is *Avanà*. Wines produced in this valley have a Protected Denomination of Origin (Valsusa VQPRD). Two vineyards of each variety were studied for three years.

### Sampling technique and sample preparation

Three hundred berries from each vineyard were picked fresh randomly at harvest. From each of these samples three samples of ten berries each were then obtained. Grape skins were placed in 25 mL of a buffer solution at pH 3.20 (12% ethanol and 300 mg/L of sodium metabisulfite) for 12 h at 20°C and then homogenised with an Ultraturrax T25 (IKA, Staufen, Germany).

### Analysis

Spectrophotometric methods were used to evaluate the total anthocyanins and flavonoid contents in the grape skins (DI STEFANO, 1996; DI STEFANO *et al.*, 1989, 1994; DI STEFANO and CRAVERO 1991).

Anthocyanins were isolated by using a SEP-PAK C18 cartridge (Waters Corporation, Milford, MA, USA) and analysed by HPLC. The chromatograph was a Perkin Elmer 200 (Norwalk, CT, USA) equipped with a 20 mL Reodyne sample loop. Data treatment was carried out using the Perkin Elmer mod. 1020 data treatment station. The analytical column was a LiChroCART (25 cm x 0.4 cm i.d.) (E. Merck, Darmstadt, Germany) packed with LiChrosphere 100 RP-18 5- $\mu$ m particles by Alltech (Decrfield, IL, USA). A Perkin Elmer LC 295 detector operating at 520 nm was used. The following conditions were used: Solvent A was 10% formic acid in water. Solvent B was 10% formic acid and 50% methyl alcohol in water. These solvents were filtered through a 0.20  $\mu$ m filter and sparged with helium. Solvent flow rate was 1 mL/min. The solvent program used was 72% A to 55% A over 15 min; to 30% A over 20 min; to 10% A over 10 min; to 1% A over 5 min; to 72% A over 3 min. An equilibrium time of 10 min was used. The single anthocyanin concentrations were determined by comparing the area of the individual peak with the total area of all the peaks and data are expressed in percentage (HEBRERO *et al.*, 1988; JAWORSKI and LEE 1987; DI STEFANO and CRAVERO, 1991).

## RESULTS AND DISCUSSION

Except for *Becouet*, total flavonoid concentrations in berry skins (Table 1) were very low in comparison to other Italian and foreign varieties such as *Cabernet franc*, *Cabernet sauvignon*, *Merlot* or

Table 1 - Phenolic composition of the four varieties.

	Avanà		Becouet		Grisa roussa		Grisa nera	
	X	$\sigma$	X	$\sigma$	X	$\sigma$	X	$\sigma$
Anthocyanins in berry skin (mg malvidin monoglucoside /kg berries)	589	49	1024	184	74	2	335	20
Flavonoids in berry skin (mg (+)catechin/kg berries)	1827	103	3772	274	1474	111	1710	106
Delphinidin-3-glucoside (%)	1.30	0.19	2.45	0.74	1.26	0.36	10.46	0.04
Cyanidin-3-glucoside (%)	22.55	5.52	0.54	0.10	94.43	0.75	19.70	2.77
Petunidin-3-glucoside (%)	3.23	0.23	4.78	1.27	0.66	0.33	10.68	0.12
Peonidin-3-glucoside (%)	50.14	2.77	3.45	0.39	1.92	0.43	14.53	0.33
Malvidin-3-glucoside (%)	16.03	3.30	36.34	5.21	0.62	0.06	28.43	2.66
Delphinidin-3-acetylglucoside (%)	0.24	0.11	0.86	0.31	0.31	0.07	0.90	0.05
Cyanidin-3-acetylglucoside (%)	0.05	0.05	0.06	0.06	0.18	0.04	1.67	0.21
Petunidin-3-acetylglucoside (%)	0.63	0.05	1.97	0.33	-	-	1.14	0.01
Peonidin-3-acetylglucoside (%)	0.11	0.04	0.74	0.05	-	-	0.97	0.05
Malvidin-3-acetylglucoside (%)	0.59	0.82	12.31	0.82	-	-	2.23	0.15
Delphinidin-3-p-coumarylglucoside (%)	0.20	0.20	4.21	0.42	-	-	1.05	0.03
Cyanidin-3-p-coumarylglucoside (%)	1.01	0.28	0.63	0.07	0.58	0.10	2.92	0.07
Petunidin-3-p-coumarylglucoside (%)	0.27	0.38	4.52	0.59	-	-	1.07	0.06
Peonidin-3-p-coumarylglucoside (%)	1.86	0.67	1.50	1.23	-	-	1.25	0.15
Malvidin-3-p-coumarylglucoside (%)	1.57	2.45	24.88	6.58	-	-	2.63	0.22
Peonidin-3-p-caffeoylglucoside (%)	0.10	0.04	-	-	-	-	0.06	0.01
Malvidin-3-p-caffeoylglucoside (%)	0.12	0.02	0.77	0.26	-	-	0.19	0.01

X: mean;  
 $\sigma$ : standard deviation;  
n = 18.

*Malbec* (ROSON and MOUTOUNET, 1992; TAMBORRA and DI BENEDETTO, 1991; UMMARINO and DI STEFANO, 1996).

The total anthocyanin concentration of the four varieties was also very low compared to 2,654 mg/kg for *Montepulciano* (TAMBORRA and DI BENEDETTO, 1991), 1,577 mg/kg for *Cabernet sauvignon*, 1,900 mg/kg for *Cabernet franc*, 2,200 mg/kg for *Merlot* or 3,100 mg/kg for *Malbec* (ROSON and MOUTOUNET, 1992). *Grisa roussa* with only 74.7 mg/kg of total anthocyanins is particularly unfavoured.

#### Avanà

The skin extract of *Avanà* mainly contained peonidin-3-glucoside and cyanidin-3-glucoside (about 73%) while malvidin-3-glucoside accounted for only 16%

of the anthocyanins. Cinnamoyl derivatives were only 6% of the anthocyanins while the acetyl derivatives were less than 2%; dihydroxylated anthocyanins were prevalent among cinnamoyl derivatives (Fig. 1).

The anthocyanin profile of *Avanà* was similar to that of *Freisa* (DI STEFANO and MAGGIOROTTO, 1995) and *Trollinger* group cultivars (WENZEL *et al.*, 1987). The *Avanà* profile was also similar to the *Nebbiolo* profile (DI STEFANO *et al.*, 1994; GUIDONI *et al.*, 1997) except for a lower amount of acylated derivatives (6-7% in *Avanà*, 10-15% in *Nebbiolo*).

#### Becouet

The berry skin extract was similar to that of *Cabernet sauvignon*, *Merlot* and *Shiraz* (NAGEL and WULF, 1979; ROGGE-

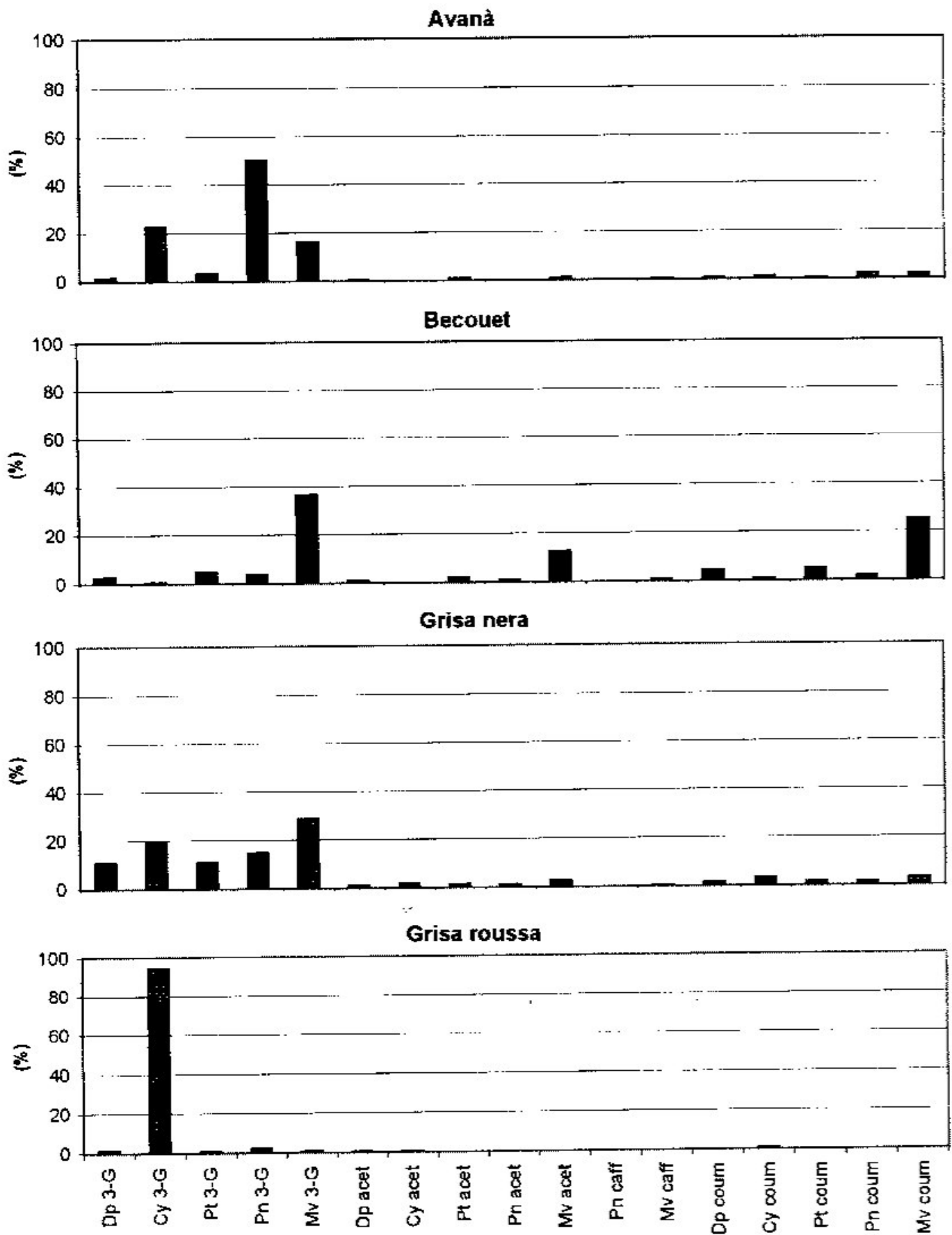


Fig. 1 - Anthocyanin profiles of the four grapevine varieties. The amount of each anthocyanin is expressed as a percentage of the total anthocyanins found in each grapevine variety. Key: Dp delphinidin; Cy cyanidin; Pt petunidin; Pn peonidin; Mv malvidin; G glucoside; acet 3-acetylglucoside; coum 3-*p*-coumarylglucoside; caff 3-*p*-caffeoylglucoside.

RO *et al.*, 1986; BOSS *et al.*, 1996). In addition to malvidin-3-glucoside (36%) and its acyl derivatives there were also large amounts of acetyl (12%) and cinnamoyl (25%) derivatives of malvidin-3-glucoside. The presence of more stable molecules, such as the trihydroxylated anthocyanins and their acylated forms, induces more stability to the wine colour during winemaking, thus the wine produced with *Becouet* is remarkably stable during winemaking and ageing. For these characteristics the *Becouet* grapes are often used to improve the colour of other wines.

#### *Grisa nera*

In the anthocyanin profile of *Grisa nera* all the unacylated anthocyanins were present in similar concentrations and malvidin-3-glucosides prevailed over cyanidin-3-glucoside and peonidin-3-glucoside. Dihydroxylated compounds were prevalent among acetyl derivatives, while the trihydroxylated were prevalent among the cinnamoyl ones. Several cultivars such as *Sangiovese* (TAMBORRA and DI BENEDETTO, 1991; WENZEL *et al.*, 1987) and *Merlot* (WENZEL *et al.*, 1987) have similar profiles.

#### *Grisa roussa*

The profile of the above mentioned cultivars were similar to other internationally well-known cultivars. On the contrary the anthocyanin profile of *Grisa roussa* is very characteristic and interesting because the non methylated form, cyanidin-3-glucoside, strongly prevailed (94.4%). The concentrations of delphinidin-3-glucoside (about 1.3%) and peonidin-3-glucoside (about 1.9%) were very low while, acetyl and cinnamoyl derivatives were nearly absent.

Other grape varieties also described have a simple anthocyanin composition, with malvidin-3-glucoside generally being the principal anthocyanin. For the red table grape *Reliance* (a hybrid *Vitis*

*vinifera* x *Vitis lambrusca*), cyanidin-3-glucoside is more than half but delphinidin-3-glucoside is nearly one third of the total anthocyanins (GAO and CAHOON, 1995). The presence of high amounts of cyanidin-3-glucoside has been reported (BOSS *et al.*, 1996; WENZEL *et al.*, 1987), but only a few cultivars with more than 90% cyanidin-3-glucoside have been reported (CRAVERO *et al.*, 1994). Generally these are cultivars with very low amounts of total anthocyanins and with no uniform colour distribution which greatly depends on the climate and ripening conditions. The pink colour of *Grisa roussa* is intense and uniform and it is not influenced by environmental conditions.

Cyanidin-3-glucoside is a very unstable molecule and in the presence of flavonoid 3'-hydroxylase and o-dihydroxyphenol-O-methyltransferase is largely transformed into the more stable pigments peonidin-3-glucoside or malvidin-3-glucoside (ROGGERO *et al.*, 1986). The absence of these stable compounds in *Grisa roussa* could be due to the absence or low activity of these enzymes or to the modified expression of UDP glucose-flavonoid 3-O-glucosyl transferase (UGFT), the enzyme responsible for the glycosylation of the various anthocyanidins in the vacuole and of the differences between colourless sports and its black-coloured progenitors (BOSS *et al.*, 1996).

Due to its very simple anthocyanin composition *Grisa roussa* can be a good tool for studies on the regulation of anthocyanin biosynthesis in grape berry skins and can provide a good source of cyanidin-3-glucoside pigment.

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