

## The anthocyanin content in berries of the hybrid grape cultivars Clinton and Isabella

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### Summary

**Berries of the hybrid grape cultivars Clinton and Isabella were analysed for their anthocyanin content and their anthocyanin profile. Anthocyanins were extracted from grape skins by an ethanol/water/hydrochloric acid solution. The extracts were used for spectrophotometric measurements and were purified by solid phase extraction to perform high performance liquid chromatography analysis (HPLC). As characteristics of hybrid grapes the anthocyanins 3,5-O-diglucoside and 3-O-(6-O-*p*-coumaroyl),5-O-diglucoside were identified. Many different anthocyanin compounds were found in Clinton grapes.**

### Introduction

In Italy a large number of hybrids can be found although the commercialization of their fruit and wine is prohibited. By permission of the European Community law, in some Italian regions cv. Isabella grapes are used for spirit production or for table grapes (G.U. of the European Community of 13/6/1998). The use of hybrid grapes can be determined by identification of specific anthocyanins which are present only in this sort of grape (HEBRERO *et al.* 1989). The qualitative and quantitative determination of grape anthocyanins can also be used to characterize grape cultivars (CALÒ *et al.* 1991, DI STEFANO 1996). In food industry, natural red pigments are used as an alternative to synthetic colorants, which needs quality control to determine the authenticity of products and to detect adulteration (HONG *et al.* 1990).

In this frame, we studied the quality and quantity of the anthocyanin compounds of the two grape cultivars.

### Material and Methods

In 1997 fruit samples were collected from the cultivars Clinton (*Vitis labrusca* x *V. riparia*) and Isabella (probably *V. vinifera* x *V. labrusca*) (FREGONI 1998) cultivated at the Istituto Sperimentale per la Viticoltura of Conegliano at Susegana (Veneto, Italy).

Anthocyanins of skins of 10 berries (17.1 g of cv. Clinton and 33.8 g of cv. Isabella) were extracted by a 25 ml ethanol/water/HCl (70:30:1 v/v/v) solution for one day in

the dark. The solution was purified by solid phase extraction (SPE) before performing HPLC analysis. To register the absorbance spectrum between 230 and 700 nm for estimation of total anthocyanin and flavonoid contents, the solution was diluted in accordance with methods reported by DI STEFANO *et al.* (1991).

Extracts were purified by SPE using a 360 mg Sep-Pak C-18 cartridge (Millipore, Waters), previously activated by washing with methanol and water. Anthocyanin profiles were registered by Varian 9010 Solvent Delivery System (Walnut Creek, CA, USA) with 50 µl loop, connected to a Varian 2550 UV-VIS detector (wavelength 520 nm). Column: LiChrospher 100 RP-18 (250 mm x 4.6 mm i.d., 5 µm) (E. Merck, Darmstadt, Germany). A Varian 4400 integrator and a WOW Chemstation software (Thermo Separation Products, Riviera Beach, FL, USA) were used. Before injection, the samples were filtered through a Nylon Acrodisc 0.2 µm filter (Gelman Sciences, Ann Arbor, Michigan, USA).

HPLC conditions: Solvent A: H<sub>2</sub>O/HCOOH (90:10 v/v); solvent B: CH<sub>3</sub>OH/H<sub>2</sub>O/HCOOH (50:40:10 v/v/v); flow rate 1 ml min<sup>-1</sup>. Gradient: from 15 % B to 45 % B over 15 min, from 45 % B to 70 % B over 30 min, from 70 % B to 90 % B over 10 min, from 90 % B to 99 % B over 5 min, from 99 % B to 15 % B over 5 min.

Total phenolics were determined by Folin-Ciocalteu reactive, proanthocyanidins (P) by extract reaction in the presence of HCl and FeSO<sub>4</sub> in a boiling water bath for 50 min, then the absorbance spectrum between 360 and 700 nm was registered. Flavans (V) were determined by reaction with vanillin. All determinations were performed according to DI STEFANO *et al.* (1991). Spectra (UV-VIS) were registered with a UVIKON 930 (Kontron Instruments S.p.A., Milan, Italy).

### Results and Discussion

The anthocyanin extraction was performed by an ethanol/water/HCl (70/30/1 v/v/v) solution to improve solubility of all anthocyanin classes. To avoid esterification, a mineral acid was used.

The HPLC chromatogram of anthocyanins of grape skin extract (cv. Clinton) is shown in the Figure. The 22 peaks of the chromatogram were identified in a preceding work by Electrospray Ionization (ESI) Mass Spectrometry analysis (FAVRETTO and FLAMINI 2000), leading to the identification of 25 compounds (Table). Among the compounds, we identified for the first time a class of anthocyanins that was found in few other hybrid cultivars: the anthocyanins delphinidin, cyanidin, petunidin and malvidin present as

3-O-(6-O-*p*-coumaroyl),5-O-diglucosides (FONG *et al.* 1974, WILLIAMS *et al.* 1978). It becomes evident that the elution order of compounds from the reversed-phase column is related to the different polarity of anthocyanins, less polar compounds being retained longer in the column (WULF *et al.* 1978). Their polarity depends on the number of glucose molecules linked to the aglycones and on the number of methoxy and hydroxyl groups present in the  $\beta$ -ring (HEBRERO *et al.* 1989). Six compounds co-eluted in pairs from the column appeared as three peaks: petunidin-3,5-O-diglucoside with delphinidin-3-O-monoglucoside (peak 3), cyanidin-3-(6-O-*p*-coumaroyl),5-O-diglucoside with cyanidin-3-O-acetylmonoglucoside (peak 12) and petunidin-3-O-acetylmonoglucoside with malvidin-3-(6-O-*p*-coumaroyl),5-O-diglucoside (peak 14).

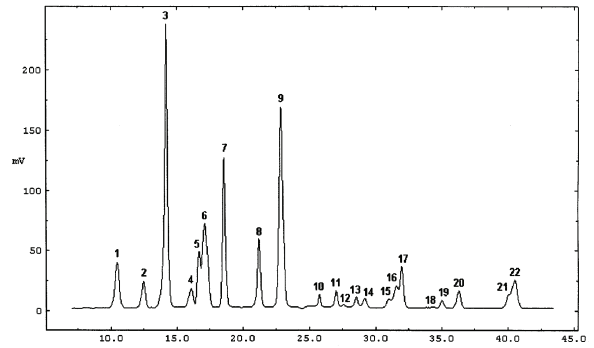


Figure: HPLC chromatogram of grape skin extracts (cv. Clinton) recorded at 520 nm. For peak identification see Table.

T a b l e

Anthocyanins in grape skin extracts of cvs Clinton and Isabella as percentage of the total anthocyanin content (as malvidin monoglucoside). Below, amounts of phenolic compounds in the skin grape extracts.  
Dp = delphinidin; Cy = cyanidin; Pt = petunidin; Pn = peonidin; Mv = malvidin; n.f. = not found

Peak	Retention time (min)	Compound	Clinton	Isabella
1	10.50	Dp-3,5-O-diglucoside	4.69	0.08
2	12.50	Cy-3,5-O-diglucoside	2.19	0.06
3	14.22	Pt-3,5-O-diglucoside+Dp-3-O-monoglucoside	22.68	10.48
4	16.08	Pn-3,5-O-diglucoside	1.93	0.98
5	16.67	Mv-3,5-O-diglucoside	5.43	6.85
6	17.14	Cy-3-O-monoglucoside	9.06	0.88
7	18.59	Pt-3-O-monoglucoside	10.99	10.03
8	21.22	Pn-3-O-monoglucoside	5.65	21.15
9	22.88	Mv-3-O-monoglucoside	20.17	33.05
10	25.78	Dp-3-O-acetylmonoglucoside	0.59	0.15
11	27.01	Dp-3-(6-O- <i>p</i> -coumaroyl),5-O-diglucoside	1.01	n.f.
12	27.52	Cy-3-(6-O- <i>p</i> -coumaroyl),5-O-diglucoside + Cy-3-O-acetylmonoglucoside	0.08	n.f.
13	28.53	Pt-3-(6-O- <i>p</i> -coumaroyl),5-O-diglucoside	0.81	n.f.
14	29.15	Pt-3-O-acetylmonoglucoside + Mv-3-(6-O- <i>p</i> -coumaroyl),5-O-diglucoside	0.83	0.09
15	30.98	Pn-3-O-acetylmonoglucoside	1.01	0.34
16	31.58	Mv-3-O-acetylmonoglucoside	2.34	0.77
17	31.97	Dp-3-O-(6-O- <i>p</i> -coumaroyl)monoglucoside	3.27	1.11
18	34.37	Mv-3-O-(6-O-caffeoyl)monoglucoside	0.08	0.38
19	35.02	Cy-3-O-(6-O- <i>p</i> -coumaroyl)monoglucoside	0.64	1.05
20	36.29	Pt-3-O-(6-O- <i>p</i> -coumaroyl)monoglucoside	1.75	1.27
21	40.11	Pn-3-O-(6-O- <i>p</i> -coumaroyl)monoglucoside	1.06	4.64
22	40.50	Mv-3-O-(6-O- <i>p</i> -coumaroyl)monoglucoside	3.74	6.61
Total diglucoside anthocyanins, %			25.58	13.21
Total monoglucoside anthocyanins, %			57.21	70.35
Total acylated anthocyanins, %			17.21	16.44
Total anthocyanins (mg Mv-monoglucoside) · kg <sup>-1</sup> fruit			1313	816
Total phenolics (mg (+)-catechin) · kg <sup>-1</sup> fruit			1818	1151
Total flavonoids (mg (+)-catechin) · kg <sup>-1</sup> fruit			2224	1800
Proanthocyanidins (P) (mg Cy-monoglucoside) · kg <sup>-1</sup> fruit			1593	1181
Flavans (V) (mg (+)-catechin) · kg <sup>-1</sup> fruit			292	311
V/P			0.18	0.26
Weight of 10 berries g			17.1	33.8

Comparing the data of skin anthocyanin contents of Clinton and Isabella grapes (Table), it can be noted that there are more different anthocyanin compounds in cv. Clinton than in cv. Isabella. In fact the latter did not contain any 3-O-(6-O-*p*-coumaroyl),5-O-diglucoside anthocyanin nor the cyanidin-3-O-acetylmonglucoside. This is in accordance with results obtained from the ESI mass spectrometry analysis. Data show that the percentage of total 3,5-O-diglucosides in extracts of cv. Clinton is almost twice as high than in extracts of cv. Isabella, where the only anthocyanin 3,5-O-diglucoside present in considerable amount was the malvidin. On the contrary, the percentage of total monoglucoside anthocyanins is higher in extracts of cv. Isabella, while the percentages of total acylated compounds are similar for both cultivars.

In the Table some parameters correlating with the phenolic composition of two grape varieties are presented. It is emphasized that concentrations of phenolic compounds, flavonoids and proanthocyanidins are higher in cv. Clinton than in cv. Isabella. The parameter V over P (V/P) was studied to estimate the abundance of flavan-3-ols (catechins and epicatechins) present as monomers in the extract: the higher the V/P value the more flavan-3-ols are present as monomers. Comparing V, P and V/P values of the two cultivars, we conclude that they have similar contents of flavan-3-ols monomers (similar values of V), while contents of procyanidins and proanthocyanidins are higher in cv. Clinton than in cv. Isabella.

## References

- CALÒ, A.; TOMASI, D.; CRAVERO, M. C.; DI STEFANO, R.; 1994: Contributo alla caratterizzazione e classificazione varietale (*Vitis* sp.), attraverso la determinazione degli antociani e degli acidi idrossicinnamoiltartarici della buccia di varietà a bacca rossa. Riv. Vitic. Enol. **3**, 13-25.
- DI STEFANO, R.; 1996: Metodi chimici nella caratterizzazione varietale. Riv. Vitic. Enol. **1**, 51-56.
- ; CRAVERO, M. C.; 1991: Metodi per lo studio dei polifenoli nell'uva. Riv. Vitic. Enol. **2**, 37-45.
- FAVRETTO, D.; FLAMINI R.; 2000: Application of Electrospray Ionization Mass Spectrometry to the Study of Grape Anthocyanins. Am. J. Enol. Vitic. **51**, 55-64.
- FONG, R. A.; WEBB, A. D.; KEPNER, R. E.; 1974: Acylated anthocyanins in a hybrid grape *Vitis* variety. Phytochemistry **13**, 1001-4.
- FREGONI, M.; 1998: Viticoltura Generale. Ed. l'Informatore Agrario S.r.l., Verona.
- HEBRERO, E.; GARCIA-RODRIGUEZ, C.; SANTOS-BUELGA, C.; RIVAS-GONZALO, J. C.; 1989: Analysis of anthocyanins by high performance liquid chromatography-diode array spectroscopy in a hybrid grape variety (*Vitis vinifera* x *Vitis berlandieri* 41B). Am. J. Enol. Vitic. **40** (4), 283-291.
- HONG, V.; WROLSTAD, R. E.; 1990: Characterization of anthocyanin-containing colorants and fruit juices by HPLC/photodiode array detection. J. Agric. Food Chem. **38** (3), 698-708.
- WILLIAMS, M.; HRAZDINA, G.; WILKINSON, M.; SWEENEY, J. G.; IACOBUCCI, G. I.; 1978: High liquid chromatographic separation of 3-glucosides, 3,5-diglucosides, 3-(6-O-*p*-coumaroyl)glucosides and 3-(6-O-*p*-coumaroylglucoside)-5-glucosides of anthocyanidins. J. Chromatogr. **155**, 389-398.
- WULF, L. W.; NAGEL, C. W.; 1978: High-pressure liquid chromatographic separation of anthocyanins of *Vitis vinifera*. Am. J. Enol. Vitic. **29** (1), 42-49.

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