EVOLUTION OF ANTHOCYANIN PROFILE FROM GRAPE TO WINE

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Abstract

Aims : This study aimed at acquiring knowledge of the evolution of anthocyanins from grape to wine and the possibility of deducing the varietal origin of a wine from its anthocyanin profile.

Methods and results : The anthocyanin « fingerprint », or profile, of a series of autochthonous Sicilian accessions and their respective young wines was determined by HPLC-DAD in 2008. Data were evaluated by taking into account the evolution of the percentages of side-ring di-oxygenated and tri-oxygenated anthocyanins, the ratio between acetylated and p-coumaroylated derivatives, and the principal component analysis (PCA) results. From grape to the end of alcoholic fermentation, the percentages of 3-glucosides of cyanidin, peonidin, delphinidin and p-coumaroylglucosides decreased, whereas those of malvidin-3-glucoside, acetylated derivatives and, in some cases, petunidin-3-glucoside increased. In many but not all wines, after malolactic fermentation, the percentages of p-coumaroylated derivatives decreased further and those of cyanidin-3-glucoside and acetylated derivatives increased ; less variation was observed in malvidin-3-glucoside values.

Conclusions : From grapes to young wines, the anthocyanin profiles varied mainly because of the activity of polyphenol oxidase (PPO), yeasts and lactic acid bacteria. However, they were substantially retained in wines from varieties in which tri-oxygenated side-ring anthocyanins prevailed, but were dramatically different from the respective grapes in wines from varieties rich in di-oxygenated anthocyanins, or in which the di-oxygenated forms prevailed over the tri-oxygenated ones. PCA analysis confirmed these findings.

Significance and impact of the study : Only in varieties in which the anthocyanin profile of grapes is characterized by low percentages of di-oxygenated side-ring forms is it possible to gain information about the varietal origin of a young wine by comparing the profiles of both grape and wine. However, great care is required.

Key words : anthocyanins, grapes, wines, anthocyanin profile, varietal origin

Résumé

Objectifs : Cette étude vise à acquérir des connaissances sur l'évolution des anthocyanes du raisin au vin et sur la possibilité de déduire le cépage d'origine d'un vin à partir de son profil anthocyanique.

Méthodes et résultats : Les profils anthocyaniques d'une série d'accessions autochtones de la Sicile et de vins jeunes respectifs ont été déterminés par HPLC-DAD en 2008. Les données ont été évaluées en tenant compte de l'évolution des pourcentages d'anthocyanes à noyau latéral di- et tri-oxygéné, du rapport entre les dérivés acétylés et p-coumaroylés ainsi que des résultats de l'analyse en composantes principales (ACP). Du raisin à la fin de la fermentation alcoolique, les pourcentages de la cyanidine, de la péonidine, de la delphinidine et des dérivés p-coumaroylés ont diminué, tandis que ceux de la malvidine-3-glucoside, des dérivés acétylés et dans certains cas, de la pétunidine-3-glucoside ont augmenté. Après la fermentation malolactique, dans un grand nombre de vins, les pourcentages des dérivés p-coumaroylés ont encore baissé, tandis que ceux de la cyanidine-3-glucoside et des dérivés acétylés ont augmenté ; une variation moindre a été observée pour la malvidine-3-glucoside.

Conclusions : Des raisins aux vins jeunes, les profils anthocyaniques ont varié principalement à cause de la polyphénol oxydase (PPO), des levures et des bactéries lactiques. Toutefois, ils ont été considérablement conservés dans les vins provenant de cépages où les anthocyanes à noyau latéral di- et tri-oxygéné (B) ont prévalu, mais ils étaient considérablement différents dans les cépages riches en anthocyanes di-oxygénés ou dans lesquelles les formes di-oxygénées ont prévalu sur les tri-oxygénées. L'analyse de l'ACP a confirmé ces résultats.

Signification et impact de l'étude : Pour les cépages où le profil anthocyanique des raisins est caractérisé par un faible pourcentage de formes à noyau latéral di- et tri-oxygéné, il est possible d'obtenir des informations sur l'origine variétale d'un vin jeune en comparant les profils anthocyaniques du raisin et du vin. Toutefois, une grande prudence est nécessaire.

Mots-clés : anthocyanes, raisins, vins, profil anthocyanique, origine variétale

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INTRODUCTION

Anthocyanins of *Vitis vinifera* are 3-glucosyl derivatives, substituted with two (di-oxygenated) or three (tri-oxygenated) -OH and -OCH_3 groups in the side-ring (B) of the flavonoid structure. Position 6 of glucose can be esterified (acylated anthocyanins) by acetic (acetylated), p-coumaric (p-coumaroylated) and, marginally, by caffeic acid (caffeoylated derivatives). The percentages of single anthocyanins in skins represent the anthocyanin « fingerprint », or profile, of the grape which, in all varieties of *Vitis vinifera*, is characterized by different ratios of di-oxygenated vs. tri-oxygenated side-ring forms, acetylated vs. p-coumaroylated derivatives, and the presence or absence of acylated derivatives. Within these three groups, there are subgroups that differ in ratios between percentages of the five non-acylated anthocyanins (Mattivi *et al.*, 1990). For example, in varieties in which di-oxygenated forms prevail over tri-oxygenated ones, cyanidin-3-glucoside or peonidin-3-glucoside may be the principal anthocyanin ; in other varieties, malvidin-3-glucoside (tri-oxygenated) is almost always the main one, but peonidin-3-glucoside (di-oxygenated) may exceed the levels of delphinidin-3-glucoside and petunidin-3-glucoside (tri-substituted forms). Among acylated derivatives, the acetylated form may prevail over the p-coumaroylated form or vice versa, or the percentage of the p-coumaroylated form may largely exceed that of acetylated derivatives (Mattivi *et al*., 1990).

Although it has been reported (Cholet and Darné, 2004 ; Castellarin *et al*., 2006) that, at veraison, the biosynthesis of di-oxygenated exceeds that of tri-oxygenated anthocyanins, in varieties in which tri-oxygenated prevail over di-oxygenated forms, the anthocyanin profile generally remains largely unchanged during ripening and is little influenced by cultural, climatic or environmental variables which may affect the anthocyanin profile in varieties in which di-oxygenated prevail over tri-oxygenated forms (Di Stefano *et al*., 1993, 1994a ; Ummarino *et al*., 2001, Di Stefano *et al*, 2002). Some of the latter varieties, e.g., Gaglioppo di Cirò (Calabria) in which cyanidin-3-glucoside prevails over other anthocyanins (Lovino *et al*., 2006), are characterized by such low percentages of acylated derivatives that sometimes they can hardly be determined. The same has been observed in varieties such as Sangiovese (Tuscany) and Nerello Mascalese (Mount Etna), in which malvidin-3-glucoside usually prevails and the percentages of other anthocyanins may be high (Di Stefano *et al*., 1994a ; Crespan *et al*, 2002). These large differences have been exploited previously for varietal characterization (Roggero *et al*, 1988 ; Mattivi *et al*, 1990 ; Garcia-Beneytez *et al*, 2003 ; De Villiers *et al*, 2004).

Determination of the varietal origin of wines is more difficult, due to possible differences between the anthocyanin profiles of grapes and wine.

In view of the importance of recognition of the varietal origin of wines, to verify literature data, and improve knowledge of this topic, in this study the evolution of anthocyanin profile from a series of grapes to their respective wines was followed.

MATERIALS AND METHODS

1. Grapes and winemaking

In 2008, determinations of the anthocyanin profile were performed in grapes of a series of accessions of autochthonous Sicilian varieties, a list of which is reported in table 1, and in the respective wines after alcoholic and malolactic fermentation. DNA microsatellite analysis was used to determine the belonging of each accession to a given cultivar (unreported data). The grapes were cultivated (as part of a clonal selection project) in the experimental vineyard of the Viticulture and Enology Research Center of the Sicilian Region Extension Service located in Marsala (Sicily), harvested from September 13 to 15 and vinified with the following standard procedure in the experimental cellar of the same Center : 40 kg of grapes of each accession were placed in plastic crates, moved to the winery, stored in a conditioned room for about 30 hours at 8 °C, destemmed, crushed, placed in stainless steel tanks (50 L), added with 0,1 g/kg diammonium phosphate (DAP), 6 mg/kg thiamine, and 0,2 g/kg reactivated dry yeast (OE Red 40, OE Italia, Marsala, Italy), and fermented at 26 °C in a conditioned room. The musts were again added with 0.1 and 0,05 g/L DAP and pumped over when reaching respectively 2 and 8 % alcohol. In addition, two punch downs were performed daily and the macerations were stopped at the end of alcoholic fermentation. Malolactic fermentation was induced by natural lactic acid bacteria. One winemaking was performed per accession. At the end of alcoholic fermentation, the composition of the wines was determined by means of a Winescan (FOSS) calibrated following EEC 2676 standard procedure (1990) for all parameters reported in table 2, except for total anthocyanins for which the instrument calibration was used.

2. Anthocyanin profiles of grapes

Extraction of anthocyanins from grape skins was performed as previously reported (Squadrito *et al.*, 2007) on three replicates of 20 berries for each accession, selected from a sample of 400 berries collected with the pedicel in the vineyard at harvest dates. In brief, the skin of each berry was separated from the pulp, placed in a plastic flask containing 25 ml of pH 3.2 tartaric buffer (5 g tartaric

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Table 1 - Accessions of autochthonous Sicilian grape cultivars examined in this work.

Accession	Cultivar	Accession	Cultivar
NCa 1	Nerello Cappuccio	NAv 1	Nero d'Avola
NCa 2	Nerello Cappuccio	NAv 2	Nero d'Avola
NCa 3	Nerello Cappuccio	NAv 3	Nero d'Avola
NCa 4	Nerello Cappuccio	NAv 4	Nero d'Avola
Ali 1	Alicante	NAv 5	Nero d'Avola
Ali 2	Alicante	NAv 6	Nero d'Avola
Ali 3	Alicante	III	still undescribed cultivar
Ali 4	Alicante	IV	still undescribed cultivar
Noc 1	Nocera	NMa 1	Nerello Mascalese
I	still undescribed cultivar	NMa 2	Nerello Mascalese
II	still undescribed cultivar	V	still undescribed cultivar
Fra 1	Frappato di Vittoria	VI	still undescribed cultivar
Fra 2	Frappato di Vittoria	VII	still undescribed cultivar
Perr 1	Perricone	VIII	still undescribed cultivar
		IX	still undescribed cultivar

Table 2 - Composition of experimental wines of autochthonous Sicilian grape accession clustered per cultivar.

		Ethanol %	pH	Titratable acidity g/L[a]	Volatile acidity g/L[b]	Total anthocyanins g/L
NCa (4)	mean ± st.dev.	10.5 ± 0.8	3.00 ± 0.1	8.1 ± 0.6	0.1 ± 0.0	222.0 ± 89.4
	min - max	9.9 - 11.5	2.9 - 3.1	7.2 - 8.5	0.1 - 0.1	137.2 - 343.7
Ali (4)	mean ± st.dev.	15.6 ± 0.8	3.1 ± 0.1	6.7 ± 0.9	0.2 ± 0.1	263.4 ± 87.8
	min - max	14.4 - 16.3	2.9 - 3.3	5.9 - 8.0	0.1 - 0.3	215.1 - 394.3
Noc (1)	mean	10.4	2.8	9.7	0.2	201.3
I (1)	mean	12.0	3.0	7.1	0.2	491.2
II (1)	mean	12.1	3.1	7.2	0.3	422.1
Fra (2)	mean ± st.dev.	12.2 ± 0.9	3.3 ± 0.0	6.8 ± 0.2	0.2 ± 0.0	238.2 ± 60.8
	min - max	11.6 - 12.8	3.3 - 3.4	6.6 - 6.9	0.2 - 0.2	195.2 - 281.6
Perr (1)	mean	10.3	3.3	6.9	0.1	505.6
NAv (6)	mean ± st.dev.	12.5 ± 1.1	3.1 ± 0.1	8.0 ± 0.6	0.2 ± 0.0	437.8 ± 116.8
	min - max	10.9 - 14.2	3.0 - 3.2	7.0 - 8.6	0.1 - 0.2	335.4 - 627.8
III (1)	mean	12.5	3.4	5.5	0.1	387.6
IV (1)	mean	14.2	3.4	5,7	0.2	227.8
NMa (2)	mean ± st.dev.	11.6 ± 1.8	3.1 ± 0.2	7.6 ± 1.6	0.1 ± 0.0	287.3 ± 29.7
	min - max	10.3 - 12.9	2.9 - 3.3	6.5 - 8.7	0.1 - 0.1	266.3 - 308.5
V (1)	mean	12.5	3.2	7.5	0.1	125.4
VI (1)	mean	10.2	3.1	6.5	0.1	88.6
VII (1)	mean	10.4	3.3	6.1	0.1	90.2
VIII (1)	mean	14.5	3.4	5.7	0.2	302.7
IX (1)	mean	14.7	3.5	5.9	0.3	425.1

[a]as tartaric acid - [b]as acetic acid

NCa : Nerello Cappuccio (4) ; Ali : Alicante (4) ; Noc : Nocera (1) ; Fra : Frappato di Vittoria (2) ; Perr : Perricone (1) ; NAv : Nero d'Avola (6) ; NMa : Nerello Mascalese (2) ; I (1) ; II (1) ; III (1), IV (1), V (1), VI (1), VII (1), VIII (1) and IX (1). Number of accessions per cultivar are in brackets.

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acid, 22.2 ml 1 M NaOH, 2 g $Na_2S_2O_5$, 125 mL 95 % ethanol to 1 L with water in a volumetric flask) and stored at - 20 °C until analysis. These samples were then kept at room temperature for 4 hours, homogenized and centrifuged at 4,000 rpm, and the liquid phases were recovered in 50 mL volumetric flasks. The resulting pellets were suspended in 20 mL of pH 3.2 tartaric buffer, allowed to stand for 30 min at room temperature and then centrifuged at 4,000 rpm. The combined liquid phases were brought to 50 mL with the same buffer. For HPLC analysis, total extracts were acidified with 1 M H_3PO_4 (4.5 mL extract + 0.5 mL 1 M H_3PO_4), centrifuged (10,000 rpm) and placed in a 1.5 mL vial in an automatic sampler for injection. Previous experiments on extracting total grape skin anthocyanins by this method gave an 80-90 % recovery rate, depending on variety (Squadrito *et al.*, in prep.).

3. Anthocyanin profiles of wines

Wine anthocyanins were pre-concentrated on C_{18} Sep Pak 400 mg cartridge Waters (part n° WAT036810). A volume of wine sufficient to obtain, after pre-concentration, an anthocyanin sample of 100-200 mg/L for HPLC analysis was diluted with 5 x 10^{-3} M H_2SO_4 to an ethanol content of 3-4 % and passed through the C_{18} cartridge, previously activated in sequence with 2 mL of methanol and 3 mL of 5 x 10^{-3} M H_2SO_4. After removal of hydrophilic substances by washing with 2 mL of 5 x 10^{-3} M H_2SO_4 and cartridge dehydration, anthocyanins were recovered with methanol, collected in a 1.5 mL vial from the first colored drop (about 0.5 mL methanol), diluted with an equal volume of solvent A (10 % HCOOH/90 % H_2O v/v) and placed in the automatic sampler for HPLC injection.

All solvents and reagents were purchased from VWR International (Milan, Italy).

Anthocyanins were identified on the basis of literature data (e.g., Wulf and Nagel, 1978) and by comparison with a sample extract of Cabernet Sauvignon grape skins and authentic standards isolated in our laboratory by preparative TLC (Di Stefano and Ciolfi, 1983). Varietal anthocyanin profiles correspond to the percentages of the areas of each peak in chromatograms recorded at 520 nm. In wines, in order to avoid erroneous identification due to the presence of pyranoanthocyanin and flavanol-ethyl-anthocyanin peaks at the retention times of acylated derivatives, only the percentages of malvidin + peonidin-3-acetyl-glucosides and malvidin + peonidin-3-p-coumaroyl-glucosides (the main acylated derivatives clearly identifiable in HPLC-DAD) as well as the ratio (malvidin + peonidin-3-acetyl-glucosides) vs. (malvidin + peonidin-3-p-coumaroyl-glucosides) were examined. Conversely, in grapes, the peaks of individual acylated anthocyanins, when present, were clearly identifiable, and the ratios of acetylated vs. p-coumaroylated derivatives were easy to determine. In order to compare the anthocyanin profiles of grapes with those of their respective wines, the percentages of the same acylated derivatives were taken into account.

For HPLC analysis of anthocyanins, an Agilent 1100 liquid chromatograph equipped with a DAD detector and an Alltech 250 mm x 4.6 mm Econosphere C_{18} 5 µm (part n° 70066) column were used. The mobile phase was a linear gradient of HCOOH :CH_3OH :H_2O (10:40:50 v :v :v ; solvent B) in HCOOH :H_2O (10:90 v :v ; solvent A) (Wulf and Nagel, 1978), the flow rate was 0.48 mL/min, and the oven temperature was 45 °C. The proportions of solvent B were as follows : isocratic for 15 min with 45 % ; 15-35 min, 45-70 %; 35-45 min, 70-90 %; 45-50 min, 90-99 %; isocratic 50-54 min, 99 %; 54-56 min, 99-45 %. Detection was performed at 520 nm.

PCA component analysis was performed with the SPSS version 13 software package.

RESULTS

1. Composition of wines

From the considerable differences in alcohol and titratable acidity of the wines (table 2) it can be deduced that the period in which the grapes of each accession and cultivar reached the same sugar content was very different. Nevertheless, the fact that they were harvested on the same date for the objective of a clonal selection project did not affect the results of our experiments because the objective here was to determine the possible variations of anthocyanin profile from grape to wine. On the other hand, in previous experiments in Western Sicily we found that the varietal anthocyanin profile was quite stable during grape maturation in a particular year and terroir (Corona *et al.*, in prep.).

2. Anthocyanin profiles of grapes and wines after alcoholic and malolactic fermentation

In the accessions of Nerello Cappuccio (NCa), Alicante (Ali), Nocera (Noc) and autochthonous varieties I and II (still undescribed), tri-oxygenated anthocyanins, particularly malvidin-3-glucoside, prevailed over di-oxygenated forms (table 3a). The percentage of p-coumaroylated derivatives was very high and, in some accessions, exceeded that of malvidine-3-glucoside itself. In all accessions, the ratio of acetylated vs. p-coumaroylated derivatives was much lower than 1. After alcoholic fermentation, percentages of malvidin-3-glucoside and, in the majority of accessions, acetylated derivatives increased, and those of di-oxygenated anthocyanins and p-coumaroylated derivatives dramatically decreased ; the behavior of delphinidin and

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petunidin 3-glucosides was less clear. After malolactic fermentation (MLF), in almost all accessions cyanidin-3-glucoside and acetylated derivatives increased and p-coumaroylated derivatives decreased. Nevertheless, the evolution of all anthocyanins was characterized by great variability.

In the accessions of Frappato (Fra) and Perricone (Perr) (table 3a), malvidin-3-glucoside and, to a lesser extent, peonidin-3-glucoside prevailed over the other anthocyanins, the percentages of each, including acylated derivatives, not reaching 10 %. The ratio of acetylated vs. p-coumaroylated derivatives varied from 0.19 to 0.47. From grapes to wines, a great decrease in di-oxygenated anthocyanins and an increase in malvidin-3-glucoside occurred. Acetylated derivatives increased and the ratio of acetylated vs. p-coumaroylated derivatives varied from 0.46 to 0.59. After MLF, the increase in the percentages of malvidin-3-glucoside and, in some cases, of cyanidin-3-glucoside and acetylated derivatives, and the decrease in the percentages of other anthocyanins were confirmed. The ratio of acetylated vs. p-coumaroylated derivatives ranged from 0.35 to 0.82.

In the accessions of Nero d'Avola (NAv) (Calabrese) and autochthonous cultivars III and IV (still undescribed), rich in acetylated and p-coumaroylated derivatives, malvidin-3-glucoside prevailed over other tri-oxygenated and, especially, di-oxygenated anthocyanins (table 3b). The ratio of acetylated vs. p-coumaroylated derivatives ranged from 0.40 to 1.12. After alcoholic fermentation, the pattern of non-acylated anthocyanins remained substantially similar to that of the grapes, although in most accessions a significant increase in malvidin-3-glucoside and decrease in cyanidin and peonidin 3-glucosides were noted. The increase in acetylated and the great decrease in p-coumaroylated derivatives led to an inversion in their ratios, which ranged from 1.19 to 1.9. As a consequence of MLF, although the percentages of non-acylated anthocyanins remained almost constant, those of acylated derivatives evolved differently in all accessions. Nevertheless, the ratio of acetylated vs. p-coumaroylated derivatives remained higher than in grapes.

Anthocyanins of the accessions of Nerello Mascalese (NMa) and autochthonous cultivars V, VI and VII (still undescribed) were characterized by the prevalence of malvidin-3-glucoside, high percentages of the other four non-acylated anthocyanins, and low percentages of acylated derivatives (table 3b). After alcoholic fermentation, malvidin-3-glucosides and p-coumaroylated derivatives increased, while the other anthocyanins decreased. MLF induced a further increase in the percentage of malvidin-3-glucoside, a decrease in other non-acylated derivatives and in almost all accessions of p-coumaroylated derivatives, whereas acetylated derivatives increased greatly (ratio of acetylated vs. p-coumaroylated ranged from 1.44 to 2.10).

In the accessions of autochthonous cultivars VIII and IX (still undescribed), poor in acylated derivatives (table 3b), 3-glucosides of cyanidin and peonidin prevailed over tri-oxygenated anthocyanins. In wine, malvidin-3-glucoside became the main anthocyanin, 3-glucosides of cyanidin, peonidin and delphinidin greatly decreased as did acetylated derivatives, and p-coumaroylated ones increased. After MLF, a further decrease occurred in non-acylated anthocyanins, unlike the behavior of malvidin-3-glucoside. That of acylated anthocyanins was influenced differently in VIII and IX, probably due to the random hydrolytic action of lactic acid bacteria during MLF.

DISCUSSION

From grapes to the end of alcoholic fermentation, independently of the accession, percentages of 3-glucosides of cyanidin, peonidin, delphinidin and p-coumaroylated derivatives decreased, those of malvidin-3-glucoside and, in most cases, acetylated derivatives increased, whereas petunidin-3-glucoside followed a different trend. These results match those of our previous works (Di Stefano, 1993 ; Di Stefano *et al.*, 1994b) in which we found that the anthocyanin profile of wine could substantially differ from that of grape and was affected by the anthocyanin profile of grape and differing wine-making techniques (time of maceration, amount of sulfur dioxide added, must contact with oxygen). As the percentages of 3-glucosides of cyanidin and peonidin in the must overcome that in the skins, due to their higher rate of diffusion from skins to must with respect to the other anthocyanins, their decrease (Di Stefano, 1993 ; Di Stefano *et al.*, 1994b) was attributed to their involvement in coupled oxidation-reduction reactions (Cheynier *et al.*, 1988) (just before the onset of alcoholic fermentation). On the contrary, the decrease in delphinidin-3-glucoside was ascribed partly to its involvement in coupled oxidation-reduction reactions, partly to its difficulty in diffusing from skins to must, and partly to greater adsorption by yeasts with respect to other anthocyanins (Di Stefano and Ciolfi, 1983). In some cases, the percentage of petunidin-3-glucoside decreased but less than that of delphinidin-3-glucoside ; in others, the opposite was observed. Malvidin-3-glucoside still increased, probably due to its stability regards coupled oxidation-reduction reactions and its easy extraction from grape skins during maceration. Being more resistant than the other four anthocyanins to the attack of caffeoyl tartaric acid quinone (CTAq), generated from the oxidation of CTA mediated by grape PPO, malvidin-3-glucoside can act as a nucleophilic agent and react with this o-quinone, producing a red pigment (Sarni *et al.*, 1995 ; Cheynier *et al.*, 2006) easily detected by chromatography on C_{18} RP

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Table 3a - Anthocyanin profile (%) of a series of accessions of autochthonous Sicilian grape clustered per cultivar. For the code of cultivars and number of accessions see table 2. Analysis for each accession were performed in triplicate.

	Nerello Cappuccio (4)		Alicante (4)		Nocera (1)	I (1)	II (1)	Frappato (2)		Perricone (1)
	mean (s.d.)	min-max	mean (s.d.)	min-max	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	min-max	mean (s.d.)
GRAPES										
Delfinidin-3-g	6.1 (1.2)	5.1-7.9	2.7 (1.6)	1.3-4.9	2.6 (0.1)	6.1 (0.2)	2.4 (0.1)	5.0 (3.1)	2.8-7.1	3.9 (0.1)
Cyanidin-3-g	0.5 (0.5)	0.2-1.3	0.5 (0.2)	0.2-0.7	0.3 (0.0)	0.7 (0.2)	1.6 (0.1)	4.4 (1.9)	3.5-5.3	2.8 (0.1)
Petunidin-3-g	8.5 (3.8)	5.7-14.1	4.1 (1.5)	2.6-5.9	5.6 (0.1)	7.7 (0.2)	3.9 (0.1)	7.8 (2.3)	6.1-9.4	7.3 (0.2)
Peonidin-3-g	2.0 (0.5)	1.5-2.6	3.8 (1.0)	2.4-4.7	3.4 (0.1)	6.3 (0.2)	16.4 (0.5)	21.2 (6.0)	17.0-25.4	18.8 (0.5)
Malvidin-3-g	39.0 (6.0)	34.1-47.5	59.0 (4.7)	53.9-64.6	51.6 (1.0)	50.6 (1.4)	50.5 (1.3)	53.0 (3.7)	50.4-55.7	55.0 (1.4)
Acetylated	6.1 (0.4)	5.5-6.6	2.6 (2.1)	0.1-4.9	5.3 (0.1)	4.7 (0.1)	4.6 (0.1)	2.0 (0.2)	1.9-2.1	2.0 (0.1)
p-coumaroylated	37.7 (11.7)	20.5-46.5	27.4 (8.9)	15.8-35.8	31.3 (0.6)	24.0 (0.7)	20.8 (0.6)	6.7 (3.1)	4.5-8.9	10.3 (0.3)
Acet./p-coum.	0.2 (0.1)	0.1-0.3	0.1 (0.1)	0.0-0.2	0.2 (0.0)	0.2 (0.0)	0.2 (0.0)	0.3 (0.2)	0.2-0.5	0.2 (0.0)
WINES										
Delfinidin-3-g	3.3 (0.4)	2.8-3.6	1.9 (0.6)	1.2-2.7	3.0 (0.1)	2.6 (0.1)	1.9 (0.0)	2.9 (0.7)	2.4-3.4	2.5 (0.1)
Cyanidin-3-g	0.2 (0.1)	0.1-0.3	0.2 (0.1)	0.1-0.4	0.2 (0.0)	0.2 (0.1)	0.9 (0.0)	0.4 (0.1)	0.4-0.5	0.7 (0.0)
Petunidin-3-g	7.3 (0.5)	6.6-7.6	4.3 (0.8)	3.3-5.3	6.0 (0.2)	5.4 (0.1)	4.9 (0.1)	6.7 (1.4)	5.8-7.7	6.2 (0.2)
Peonidin-3-g	1.3 (0.6)	0.7-2.0	2.6 (0.7)	1.8-3.4	2.6 (0.1)	4.8 (0.1)	7.9 (0.2)	5.4 (0.2)	5.3-5.5	10.0 (0.2)
Malvidin-3-g	69.9 (0.4)	69.6-70.4	74.7 (1.4)	72.6-75.8	67.0 (1.7)	61.2 (1.4)	66.7 (1.5)	73.3 (0.1)	73.2-73.3	69.5 (1.7)
Acetylated	7.3 (1.0)	6.1-8.6	4.5 (0.7)	3.8-5.4	4.3 (0.1)	8.2 (0.2)	7.5 (0.2)	3.9 (0.4)	3.7-4.2	3.5 (0.1)
p-coumaroylated	10.7 (0.5)	10.0-11.1	11.8 (1.6)	10.2-14.1	17.0 (0.4)	17.8 (0.4)	10.9 (0.2)	7.4 (1.7)	6.2-8.6	7.5 (0.2)
Acet./p-coum.	0.7 (0.1)	0.5-0.8	0.4 (0.1)	0.3-0.5	0.3 (0.0)	0.5 (0.0)	0.7 (0.0)	0.5 (0.1)	0.5-0.6	0.5 (0.0)
WINES MLF										
Delfinidin-3-g	3.2 (0.9)	2.4-4.4	2.0 (0.6)	1.6-2.8	2.9 (0.1)	2.9 (0.1)	2.5 (0.1)	1.9 (0.3)	1.7-2.2	2.2 (0.1)
Cyanidin-3-g	0.7 (0.1)	0.6-0.8	0.9 (0.5)	0.4-1.5	1.3 (0.0)	0.4 (0.0)	0.7 (0.0)	0.7 (0.4)	0.4-1.0	0.6 (0.0)
Petunidin-3-g	7.1 (0.8)	6.2-8.1	4.2 (0.8)	3.4-5.3	6.4 (0.2)	5.7 (0.1)	4.9 (0.1)	5.3 (0.3)	5.1-5.5	6.2 (0.2)
Peonidin-3-g	1.4 (1.0)	0.8-2.8	2.8 (0.7)	1.8-3.3	1.5 (0.0)	5.3 (0.1)	8.8 (0.2)	5.2 (0.8)	4.7-5.7	8.6 (0.2)
Malvidin-3-g	68.1 (1.5)	66.2-69.7	75.3 (2.7)	72.7-78.0	57.3 (1.6)	59.9 (1.2)	66.4 (1.4)	76.4 (2.5)	74.6-78.1	71.0 (1.2)
Acetylated	9.6 (2.5)	6.0-11.7	4.6 (2.6)	3.1-8.5	14.8 (0.4)	11.7 (0.3)	5.2 (0.1)	3.5 (1.5)	2.5-4.6	5.1 (0.1)
p-coumaroylated	10.0 (1.6)	8.3-12.0	10.2 (1.3)	8.3-11.3	15.8 (0.4)	14.1 (0.3)	11.5 (0.3)	7.1 (0.1)	7.0-7.1	6.2 (0.2)
Acet./p-coum.	1.0 (0.3)	0.6-1.3	0.5 (0.4)	0.3-1.0	0.9 (0.0)	0.8 (0.0)	0.5 (0.0)	0.5 (0.2)	0.4-0.6	0.8 (0.0)

Table 3b - Anthocyanin profile (%) of a series of accessions of autochthonous Sicilian grape clustered per cultivar. For the code of cultivars and number of accessions see table 2. Analysis for each accession were performed in triplicate.

	Nero d'Avola (6)		III (1)	IV (1)	Nerello Mascalese (2)		V (1)	VI (1)	VII (1)	VIII (1)	IX (1)
	mean (s.d.)	min-max	mean (s.d.)	mean (s.d.)	mean (s.d.)	min-max	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)
GRAPES											
Delfinidin-3-g	4.7 (1.7)	3.0-7.6	3.1 (0.1)	19.4 (0.6)	17.1 (1.2)	16.2-17.9	12.5 (0.3)	10.1 (0.3)	14.7 (0.4)	14.0 (0.4)	14.4 (0.4)
Cyanidin-3-g	0.4 (0.3)	0.2-0.9	0.3 (0.0)	13.6 (0.4)	17.1 (0.2)	17.0-17.3	8.4 (0.2)	14.4 (0.4)	10.7 (0.3)	36.5 (1.0)	25.6 (0.6)
Petunidin-3-g	5.7 (1.6)	4.3-8.6	3.3 (0.1)	18.0 (0.6)	15.0 (0.1)	14.9-15.1	17.2 (0.5)	15.6 (0.4)	15.4 (0.4)	8.7 (0.3)	13.5 (0.3)
Peonidin-3-g	2.2 (1.0)	1.2-3.8	3.0 (0.1)	3.3 (0.1)	17.8 (0.3)	17.6-18.0	9.2 (0.5)	17.6 (0.4)	7.5 (0.2)	22.0 (0.6)	13.3 (0.3)
Malvidin-3-g	44.1 (3.3)	39.3-47.4	36.7 (0.8)	18.4 (0.6)	32.3 (1.1)	31.5-33.1	40.0 (0.3)	41.6 (1.0)	38.7 (0.9)	16.8 (0.4)	30.2 (0.8)
Acetylated	14.1 (0.9)	12.6-15.0	22.4 (0.6)	14.4 (0.5)	0.0 (0.0)	0.0-0.0	4.6 (0.1)	0.0 (0.0)	2.7 (0.1)	0.8 (0.0)	0.6 (0.0)
p-coumaroylated	28.8 (6.5)	19.2-36.9	31.2 (0.8)	12.8 (0.4)	0.7 (0.0)	0.7-0.8	8.1 (0.2)	0.7 (0.0)	10.3 (0.3)	1.3 (0.0)	2.4 (0.1)
Acet./p-coum.	0.5 (0.1)	0.4-0.7	0.7 (0.0)	1.1 (0.0)	0.0 (0.0)	0.0-0.0	0.6 (0.0)	0.0 (0.0)	0.3 (0.0)	0.6 (0.0)	0.3 (0.0)
WINES											
Delfinidin-3-g	3.4 (1.0)	2.1-4.6	1.4 (0.0)	10.2 (0.2)	11.3 (3.9)	8.5-14.0	6.1 (0.2)	10.4 (0.3)	2.6 (0.1)	9.4 (0.2)	6.7 (0.2)
Cyanidin-3-g	0.2 (0.1)	0.1-0.2	0.2 (0.0)	1.3 (0.0)	2.8 (1.8)	1.6-4.1	0.7 (0.0)	1.5 (0.0)	0.3 (0.0)	6.9 (0.2)	2.8 (0.1)
Petunidin-3-g	6.3 (1.0)	4.8-7.5	3.3 (0.1)	13.5 (0.3)	15.9 (1.5)	14.9-17.	10.1 (0.3)	13.9 (0.4)	6.2 (0.1)	13.0 (0.3)	13.7 (0.3)
Peonidin-3-g	1.5 (0.5)	0.8-2.2	2.0 (0.1)	1.6 (0.0)	10.5 (2.2)	9.0-12.1	2.9 (0.1)	5.5 (0.1)	2.0 (0.0)	16.7 (0.4)	6.8 (0.2)
Malvidin-3-g	63.1 (2.3)	58.5-64.8	50.8 (1.2)	45.6 (1.1)	57.9 (10.1)	50.7-65.0	54.7 (1.5)	61.2 (1.5)	70.6 (1.6)	50.7 (1.3)	65.0 (1.4)
Acetylated	15.4 (0.7)	14.3-16.2	23.1 (0.6)	15.6 (0.4)	0.0 (0.0)	0.0-0.0	15.0 (0.4)	3.2 (0.1)	8.2 (0.2)	0.0 (0.0)	0.0 (0.0)
p-coumaroylated	10.2 (1.6)	7.8-12.4	19.4 (0.5)	12.2 (0.3)	1.5 (0.7)	1.0-2.0	10.5 (0.3)	4.4 (0.1)	10.2 (0.2)	3.3 (0.1)	5.0 (0.1)
Acet./p-coum.	1.5 (0.2)	1.0-1.9	1.2 (0.0)	1.3 (0.0)	0.0 (0.0)	0.0-0.0	1.4 (0.0)	0.7 (0.0)	0.8 (0.0)	0.0 (0.0)	0.0 (0.0)
WINES MLF											
Delfinidin-3-g	3.3 (1.1)	1.5-4.9	1.1 (0.0)	6.7 (0.2)	7.2 (3.7)	4.6-9.8	6.2 (0.2)	7.7 (0.2)	1.8 (0.1)	2.1 (0.1)	4.7 (0.1)
Cyanidin-3-g	0.3 (0.2)	0.1-0.7	0.1 (0.0)	1.2 (0.0)	2.5 (1.7)	1.3-3.8	0.6 (0.0)	1.7 (0.0)	0.9 (0.0)	0.7 (0.0)	2.8 (0.1)
Petunidin-3-g	5.8 (0.9)	4.4-7.0	2.9 (0.1)	11.5 (0.3)	13.4 (3.5)	10.9-15.8	10.4 (0.3)	12.7 (0.3)	4.7 (0.1)	5.6 (0.2)	12.9 (0.3)
Peonidin-3-g	1.4 (0.2)	1.2-1.8	2.3 (0.1)	1.8 (0.1)	10.5 (2.5)	8.7-12.3	2.4 (0.1)	5.5 (0.1)	1.6 (0.0)	5.9 (0.2)	7.0 (0.2)
Malvidin-3-g	66.7 (5.1)	57.4-71.0	56.8 (1.4)	50.1 (1.3)	60.8 (9.2)	54.3-67.3	61.4 (1.5)	64.7 (1.3)	70.7 (1.6)	76.1 (1.8)	71.0 (1.5)
Acetylated	12.9 (3.7)	9.6-19.0	20.9 (0.6)	17.5 (0.5)	3.4 (1.4)	2.3-4.4	11.3 (0.3)	5.2 (0.1)	11.8 (0.3)	3.3 (0.1)	0.0 (0.0)
p-coumaroylated	9.7 (1.0)	8.7-11.7	16.0 (0.5)	11.5 (0.3)	2.2 (0.8)	1.6-2.8	7.7 (0.2)	2.5 (0.1)	8.4 (0.2)	6.4 (0.2)	1.7 (0.0)
Acet./p-coum.	1.3 (0.4)	1.0-2.0	1.3 (0.0)	1.5 (0.0)	1.5 (0.1)	1.4-1.6	1.5 (0.0)	2.1 (0.0)	1.4 (0.0)	0.5 (0.0)	0.0 (0.0)

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(unpublished data). The increase in acetylated derivatives in accessions in which tri-oxygenated anthocyanins prevailed was attributed both to their good extractability from grape skins during maceration and to the fact that they are mainly derivatives of malvidin-3-glucoside, the least involved anthocyanin in coupled oxidation-reduction reactions. According to Di Stefano *et al.* (1994b) and Revilla *et al.* (2009), the decrease in p-coumaroylated derivatives (although they are mainly derivatives of malvidin-3-glucoside) is due to their very low extractability that is not counterbalanced by high stability in the above reactions. As a consequence, in these accessions, the ratio of acetylated vs. p-coumaroylated derivatives usually increased. Taking into account the increase in acetylated derivatives and the decrease in p-coumaroylated ones from grape to wine, a ratio of acetylated vs. p-coumaroylated derivatives higher than 3 would be necessary to deduce the varietal origin of Cabernet Sauvignon wines (Holbach *et al.* 2001). This value was confirmed by Otteneder *et al.* (2004), but was challenged by Burns *et al.* (2002), who found values ranging from 1.2 to 6.5.

Our results partially match earlier works in which the determination by TLC on Kieselgel and remission spectrophotometry detection of anthocyanins in grape and the respective wines of Germany (Lay and Draeger, 1991) proved that, in wines made from grapes rich in acylated and non-acylated malvidin-3-glucosides (Heroldrebe grape variety), the anthocyanin profile of grapes was retained (apart from an increase in malvidin-3-glucoside and a decrease in other acylated and non-acylated derivatives). In wines made from grapes rich in cyanidin and peonidin 3-glucosides and poor in acylated derivatives (Helfensteiner grape variety), the anthocyanin profile differed substantially from that of the respective grapes, in that malvidin-3-glucoside became the main anthocyanin present. In Pinot noir (Gao *et al.*, 1997), a variety lacking acylated anthocyanins, comparisons between anthocyanin profiles of grape and wine showed a strong decrease in peonidin-3-glucoside and changes in the ratios among anthocyanins other than malvidin-3-glucoside.

In grapes of the accessions in which the di-oxygenated anthocyanins prevailed over the tri-oxygenated ones and the percentages of acylglucosides of cyanidin and peonidin were higher than those of malvidin-3-glucoside (as acetylated derivatives were extracted from the skins faster than the p-coumaroylated ones, when PPO were more active), we found that cyanidin and peonidin acetylglucosides, being involved in coupled oxidation-reduction reactions, were more oxidized than p-coumaroylglucosides. The percentage of acetylated derivatives consequently decreased, whereas that of p-coumaroylglucosides increased.

After malolactic fermentation, in most cases, the percentages of p-coumaroylated derivatives further decreased, those of cyanidin-3-glucoside acetylated derivatives (in about two-thirds of the wines) and malvidin-3-glucoside (in almost all wines) increased, and those of delphinidin and petunidin 3-glucosides showed good stability. The decrease in p-coumaroylated derivatives may be explained as due to the hydrolytic activity of lactic acid bacteria against hydroxycinnamoyl tartaric acids (Hernández *et al.*, 2007). Acetylated derivatives are evidently more stable than p-coumaroylated ones toward these enzymatic hydrolytic reactions. However, the variability induced by the bacterial strains responsible for MLF don't allow these findings to be generalized. Also anthocyanase activity of lactic acid bacteria (Vivas *et al.*, 1995) and degradation reactions via calcone (Guerra, 1997) may have been responsible for the variations in anthocyanin profile found after MLF. The increase in percentage of cyanidin-3-glucoside after MLF may be explained by its greater stability with respect to other anthocyanins to hydrolytic degradation into the calcone form, due to lesser activation of the side-ring. The good stability of malvidin-3-glucoside in MLF may be ascribed to its lesser involvement than other anthocyanins in enzymatic oxidation reactions which probably continued, although to a modest extent, at the same time as esterase and anthocyanase activity of lactic acid bacteria. However, the possibility that some anthocyanins were selectively adsorbed by bacterial cells cannot be excluded. In this case again, the variability of results obtained in individual vinifications does not allow us to draw general conclusions and requires further investigation.

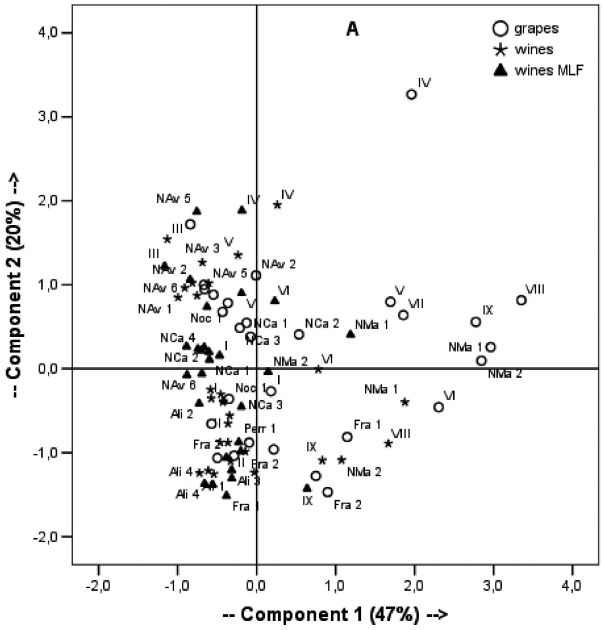
The above problems increase when the varietal origin of aged wines has to be determined, because of the changes occurring in the anthocyanin profiles of wine during storage (Dallas *et al.*, 1995). According to McCloskey and Yengoyan (1981), acylated derivatives degrade faster than non-acylated ones, and acetylated more than p-coumaroylated ones.

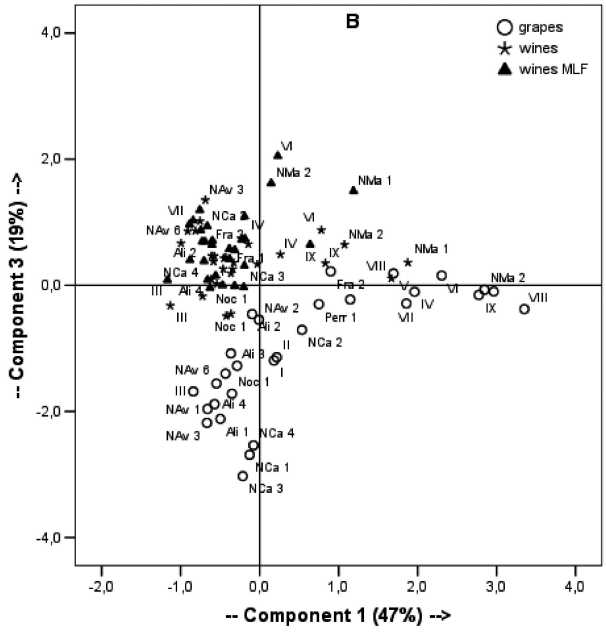
Principal component analysis

In PCA, the first three components explained 86.4 % of total variance : the first component accounted for 47.2 %, and was highly correlated with non-acylated anthocyanins and acetylated derivatives ; the second, at 20.2 %, was highly correlated with acetylated derivatives and the ratio of acetylated vs. p-coumaroylated ones ; and the third, at 19.1 %, was highly correlated with p-coumaroylated derivatives.

The plot displaying PC1 vs. PC2 (figure 1, plot A) showed that only in some of the accessions in which tri-oxygenated anthocyanins prevailed and the ratio of acetylated vs. p-coumaroylated derivatives was high, did the respective wines after alcoholic and MLF occupy

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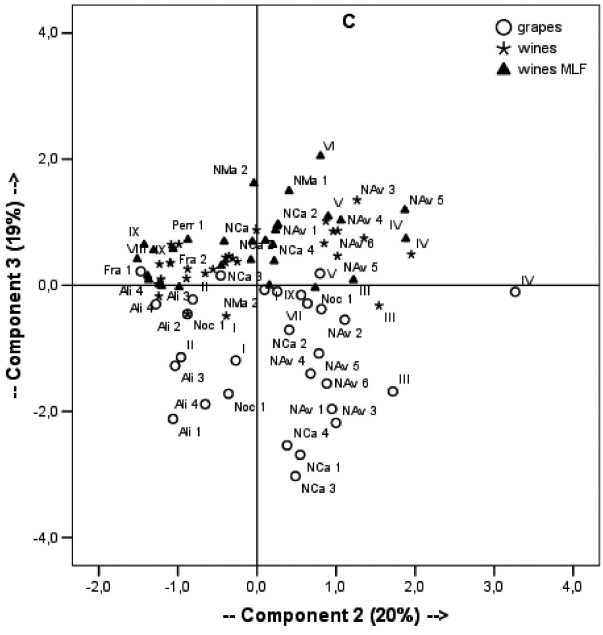


Figure 1 - PCA analysis performed on the data of anthocyanins of grape and wine of each accession listed in table 1 : plot of PC1 vs. PC2 (A), plot of PC1 vs. PC3 (B) and plot of PC2 vs. PC3 (C).

positions near those of the grapes (see negative semi-axis of PC1). In most of the accessions, the opposite positions of grapes and their corresponding wines indicated that the anthocyanin profiles of grapes and corresponding wines were very different. In these accessions, in which the percentages of di-oxygenated anthocyanins were high and those of acylated derivatives were low, it did not seem possible to deduce the original varieties from the anthocyanin profiles of the corresponding wines. The clearer separation of grapes from wines shown in plots PC1 vs. PC3 and PC2 vs. PC3 (figure 1, plots B and C) confirms that only in some cases the anthocyanin profile of a wine is similar to that of its corresponding grape.

CONCLUSIONS

The data presented in this work on the evolution of anthocyanins from grapes to wine, sufficiently confirm those of international reports and our own previous works, although important data which complete and clarify earlier data were also acquired. In real vinifications, in which PPO and coupled oxidation-reduction reactions were only partly inhibited, the anthocyanin profiles of grapes were essentially retained only in wines derived from varieties in which tri-oxygenated anthocyanins prevailed ; in the others, significant changes were observed. Almost all varieties showed a decrease in percentages of 3-glucosides of cyanidin, peonidin and delphinidin, and an increase in malvidin-3-glucoside and the ratio of acetylated vs. p-coumaroylated derivatives after alcoholic fermentation. Nevertheless, in some accessions richer in di-oxygenated anthocyanins, a decrease in the ratio of acetylated vs. p-coumaroylated derivatives was observed. MLF also contributed to the change in the anthocyanin profiles of grapes, but less than in the case of alcoholic fermentation. PCA confirmed that only in some varieties rich in tri-oxygenated anthocyanins did the anthocyanin profiles of grapes and the respective wines correspond. Consequently, only in these varieties is it possible to acquire information about the varietal origin of a monovarietal wine from its anthocyanin profile, although great prudence is required.

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