Research Article

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Anthocyanin composition and extractability in berry skin and wine of *Vitis vinifera* L. cv. Aglianico

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Abstract

BACKGROUND: The present article reports the anthocyanin content in the berry skin and wine of the Italian red grape cultivar Aglianico (clone VCR11 grafted onto 1103 Paulsen), one of the most ancient vines and famous for its deep-red colour. Anthocyanins were extracted from frozen berry skin in an acidified methanol solution. The extraction mixtures, monitored for 120 h, were analysed by high-performance liquid chromatography.

RESULTS: The extraction from berry skin of delphinidin, petunidin and malvidin appeared to be a time-independent process, whereas the concentration of peonidin increased linearly with time. Peonidin-*O*-acetyl-glucoside was transferred from skin more slowly than petunidin-*O*-acetyl-glucoside and malvidin-*O*-acetyl-glucoside. The anthocyanin composition of the resulting wine showed that the total anthocyanin content was about one-tenth of the corresponding berry skin content. The ratio acetyl/coumaroyl anthocyanins in the wine was sharply higher than the value in berry skin (0.85 and 0.10, respectively), indicating an enrichment of acetyl derivatives in the wine.

CONCLUSION: Levels of single anthocyanins in wine were not always correlated with those detected in grapes, as they were affected by winemaking. The high values of some anthocyanins in Aglianico wine could ameliorate its quality, increasing the chromatic properties, aging stability and product acceptance.

Keywords: anthocyanins; Aglianico; antioxidants; Italian grapevine; red wine composition; wine quality

INTRODUCTION

Anthocyanins are a very large group of red–blue polyphenol plant pigments, located within vacuoles of the skin cells of red grape berries, characterized by a positive charge on the molecule[1,2] (molecular formula in Table 1). These natural pigments are glycosides having a sugar, usually glucose, bonded to the C-3 position (anthocyanin = anthocyanidin aglycon + sugar). The presence of this sugar determines an increase in water solubility and allows anthocyanins to diffuse into the must and wine during maceration, contributing to the colour intensity and properties of red wines.[3,4]

Based on the position of hydroxyl and methoxyl groups on the second phenyl ring, anthocyanin pigments are classified into five groups: cyanins, petunins, peonins, malvins and delphinins.[5] Blueness and redness levels of these compounds are directly related to the number of free hydroxyl groups of methoxy groups.[6] An example of this phenomenon is malvidine, the main anthocyanin of red grapes, which has the greatest degree of methylation and thus the reddest colour. Furthermore, the presence and concentration of each of the five groups of anthocyanins are also a function of the environmental conditions and viticultural practices.[7,8] The proportion of five classes has a significant impact on the colour and colour stability of a red wine.[9] Moreover, the pattern of anthocyanin accumulation appears to be closely related to genetic characteristics of the grape itself, and these pigments can be considered as chemical markers to differentiate grape cultivars.[9]

Anthocyanins have strong anti-inflammatory properties.[10] These compounds are used to fight coronary heart disease[11] and they are a dietary supplement for the prevention and treatment of metabolic disorders, in particular obesity, weight gain, insulin

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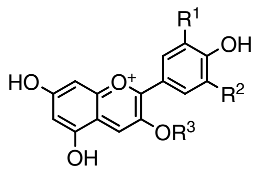
Table 1. Anthocyanins detected in the berry skin of Aglianico grapes



	Anthocyanin	Abbreviation	R^1	R^2	R^3
1	Delphinidin	Del	OH	OH	Glucose
2	Petunidin	Pet	OCH_3	OH	Glucose
3	Malvidin	Mal	OCH_3	OCH_3	Glucose
4	Peonidin	Peo	OCH_3	H	Glucose
5	Petunidin-*O*-acetyl-glucoside	Pet-acetyl	OCH_3	OH	Acetyl-glucose
6	Peonidin-*O*-acetyl-glucoside	Peo-acetyl	OCH_3	H	Acetyl-glucose
7	Malvidin-*O*-caffeoyl-glucoside	Mal-caffeoyl	OCH_3	OCH_3	Caffeoyl-glucose
8	Malvidin-*O*-acetyl-glucoside	Mal-acetyl	OCH_3	OCH_3	Acetyl-glucose
9	Petunidin-*O*-*cis*-*p*-coumaroyl-glucoside	Pet-*cis*-*p*-coumaroyl	OCH_3	OH	*cis*-*p*-Coumaroyl-glucose
10	Peonidin-*O*-*trans*-*p*-coumaroyl-glucoside	Peo-*trans*-*p*-coumaroyl	OCH_3	H	*trans*-*p*-Coumaroyl-glucose
11	Malvidin-*O*-*trans*-*p*-coumaroyl-glucoside	Mal-*trans*-*p*-coumaroyl	OCH_3	OCH_3	*trans*-*p*-Coumaroyl-glucose

resistance syndromes, diabetes, fasting hyperlipidaemia and osteoarthritis.[12,13] Cyanidin-3-glucoside, delphinidin-3-glucoside and pelargonidin-3-galactoside are the major anthocyanins that promote insulin secretion (1.4-fold at 4 mmol L^{-1} glucose concentration).[14,15] In red wines, free anthocyanins are the main constituents responsible for total antioxidant capacity.[16,17] Glycosylated and methoxy derivatives of anthocyanins, like malvidin-3-glucoside, are most effective as inhibitors at both the initiation and promotion propagation stages of tumour promotion–carcinogenesis, and protect cells against oxidative damage.[18] These antioxidant properties are due to the flavylium ion and the anthocyanin aglycon group (Fig. 1).

On this basis, this paper aims to investigate the presence and composition of anthocyanins in berry skin of the red grapevine (*Vitis vinifera* L.) cultivar Aglianico, a rarely studied but diffuse grape variety. The anthocyanin levels of grape berry skin are also compared with those of the resulting wine.

MATERIALS AND METHODS

Experimental vineyard and plant material

The experiment was carried out in 2008 on a 5-year-old vineyard of Aglianico (VCR11) grafted onto 1103 Paulsen and located in Montegiordano Marina (42° 02′N, 16°35′E; southern Italy). According to the Winkler classification,[19,20] this production area falls within climatic region 5: *very hot*. During the experiment, temperatures ranged between 0 and 38.5 °C, while cumulative rainfall over the period was 245 mm. The experimental plot, of about 0.20 ha, consisted of plants spur-pruned to a permanent horizontal unilateral cordon at about 0.60 m above the ground, and was characterized by about eight spurs of two to three buds each. The distance between the plants was 2.5 × 1.0 m, with a total plant density of 4000 plants ha^{-1}. The rows were oriented in a north–south direction. The soil was classified as a clay-loam.

The plants were weekly irrigated from 9 June to 1 August (from fruit set to veraison) using an amount of water equal to 50% of cultural evapotranspiration (ET_c). The irrigation amount of 50% of ET_c was considered the optimal value for grapevine.[21,22]

Plant water status was determined throughout the experimental period on 10 plants per treatment by measurements of stem water potential (ψ_w). The values of ψ_w were measured at 13 : 00 h on five fully expanded leaves and well lighted, selected from each plant on fruiting shoots situated in the median zone of the plant using a pressure chamber (model 600, PMS Instrument Co., Corvallis, OR, USA).

Anthocyanin extraction from berry skin and wine

At harvest, on 27 September 2008, three clusters per plant were randomly sampled in the central and well-irradiated area of the canopy of 10 plants located in the central part of the row. Berries with a weight between 0.60 and 1.25 g – the more abundant and representative weight class in the grape clusters – were detached from each cluster, peeled with a scalpel and the skin rapidly frozen at −80 °C, and were stored for analytical determination. Five grams of skin of frozen grape berries were collected and extracted at different times in a 100 mL methanol–HCl 0.75% (w/w) solution at room temperature.[23,24] The extraction was monitored for 120 h. Eleven bottles of 1-year-old wine, made from the reported grapes, were examined, and the wine content was analysed by high-performance liquid chromatography (HPLC) for anthocyanin determination.

Anthocyanin separation, identification and quantification

The anthocyanin contents of wine and skin extracts were determined by HPLC (HP 1110, Agilent Technologies, Palo Alto, CA, USA). All berry skin extracts and wines were filtered through a 0.45 µm Whatman filter. All the experiments were performed in five independent replicates for berry skin and in 11 independent replicates for wine analysis.

HPLC–mass spectrometric analyses were conducted to confirm the identity of each peak using an HP 1100 MS system with a PDA UV-visible detector coupled to an Agilent 6110 quadrupole liquid chromatograph/mass spectrometer equipped with an ESI source (Agilent Technologies), a Luna 5 µm Phenyl-Hexyl column, 4.60 mm × 250 mm (Phenomenex Inc., Torrance, CA, USA) and

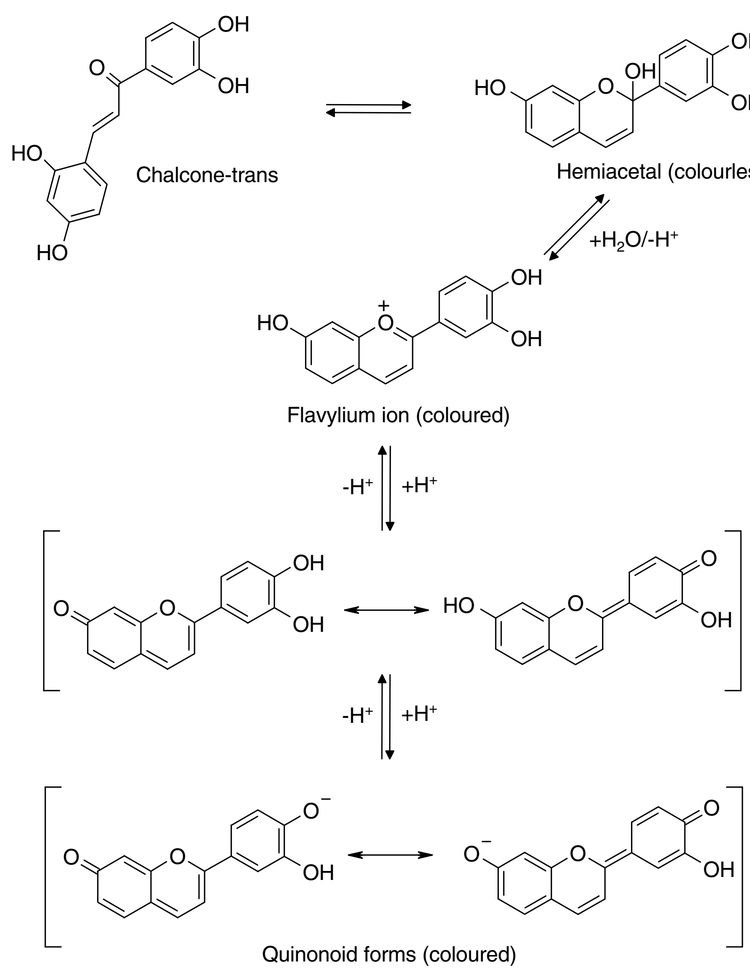


Figure 1. Equilibrium of anthocyanin structure conversion.

injection valve (20 µL loop). An aqueous solution containing 0.1% trifluoroacetic acid (TFA) and MeCN was used as eluent. The column was eluted at room temperature using a consecutive isocratic gradient of 100% aqueous solution containing 0.1% TFA for 1 min, then a gradient of 0–40% MeCN for 39 min, 40–0% MeCN for 1 min, and 100% aqueous solution containing 0.1% TFA for 1 min at a flow rate of 1.0 mL min^{-1}.

Mass scans were measured from *m/z* 100 up to *m/z* 800. Mass spectrometric data were acquired in the negative ionization mode. Anthocyanins were quantified as malvidin-3-glucoside, using malvidin-3-glucoside chloride as external standard (Extrasynthèse SAS, Genay, France).

RESULTS AND DISCUSSION

On 24 May, the value of stem water potential (ψ_w) in measured plants was -0.6 ± 0.5 MPa. At flowering (31 May), ψ_w decreased to -0.8 ± 0.4 MPa, reaching values of -1.34 ± 0.5 MPa at the beginning of July, and then remaining constant until harvest. These changes in ψ_w can be considered normal, as soil water content decreased with the increasing water evapotranspiration occurring in the summer months.

Eleven different anthocyanins, the 3-*O*-monoglucosides of Del, Pet, Peo and Mal, and some of their derivatives were detected in the extracts of berry skin of Aglianico grapes at harvest time (Table 1).

The average anthocyanin concentrations in Aglianico berry skin, expressed as mg kg^{-1} fresh weight (f.w.) of berry skin ($\pm$ standard deviation), arising from the HPLC analysis are reported in Table 2.

According to the chromatogram shown in Fig. 2, the dominant anthocyanins after a 24 h extraction were Mal (60.9%) and Mal-*trans*-*p*-coumaroyl (22.7%). The level of Mal found in Aglianico berry skin can be considered intermediate, as Mal minimum and maximum contents were found in Sangiovese (50%) and Grenache (90%), respectively,[25] whereas Mal-*trans*-*p*-coumaroyl content was particularly high if compared to other red grape varieties, such as Monastrell, Cabernet Sauvignon and Merlot.[26] In the Aglianico berry skin analysed here, the anthocyanins Del, Peo and Pet were also present together with Peo-acetyl (0.91%) and Pet-acetyl (0.74%), and remarkable amounts of Pet-*cis*-*p*-coumaroyl (1.24%) and Peo-*trans*-*p*-coumaroyl (1.08%) were also observed (Fig. 2). The percentages of Peo and Pet acetyl and coumaroyl derivatives observed here are comparable to those found in the red grape varieties Cabernet Sauvignon, Syrah and Merlot.[26] Furthermore, a small amount of Mal-caffeoyl (0.873%) was detected in berry skin (Fig. 2).

Table 2 and Fig. 3 show the presence of anthocyanins in berry skin and give a measure of their different time/extractability in methanol–HCl 0.75% (w/w). The extraction of Del, Pet, and Mal appeared to be a time-independent process, mainly due to the initial presence of these free pigments in the berry skin, whereas the concentration of Peo increased linearly in time, suggesting

Table 2. Average anthocyanin concentration (± standard deviation; $n = 5$) in the berry skin of Aglianico grapes measured at different extraction times. Abbreviations as in Table 1

	Anthocyanin	Mass (m/z)	Extraction time: 20 min	30 min	1 h	3 h	6 h	8h	24 h	30 min	24 h
			Anthocyanin content (mg kg^{-1} f.w. of berry skin)							(%)	
1	Del	465.303	296.5 ± 0.6	366.3 ± 3.1	360.0 ± 1.6	348.7 ± 1.2	310.6 ± 2.0	301.5 ± 1.2	288.2 ± 0.1	4.10	4.36
2	Pet	479.317	398.9 ± 0.8	430.2 ± 1.6	427.9 ± 0.9	438.9 ± 0.7	384.9 ± 1.4	386.1 ± 1.6	355.5 ± 0.1	4.80	5.38
3	Mal	493.381	4660.4 ± 3.9	5569.4 ± 3.6	4816.0 ± 2.5	4829.6 ± 1.4	4398.2 ± 0.8	4341.2 ± 2.3	4024.9 ± 2.6	62.4	60.9
4	Peo	463.301	10.8 ± 1.1	33.9 ± 1.0	36.5 ± 0.2	37.7 ± 0.6	52.9 ± 1.2	53.7 ± 1.6	53.8 ± 0.1	0.380	0.662
5	Pet-acetyl	521.170	26.5 ± 0.1	52.8 ± 1.6	53.0 ± 1.2	40.8 ± 0.9	69.7 ± 0.1	66.4 ± 0.8	49.0 ± 2.3	0.592	0.741
6	Peo-acetyl	505.301		66.2 ± 0.3	58.2 ± 0.6	56.6 ± 1.2	75.8 ± 0.4	69.7 ± 1.0	60.4 ± 0.7	0.742	0.913
7	Mal-caffeoyl	655.331	68.0 ± 1.0	77.9 ± 0.1	77.5 ± 0.7	74.8 ± 0.7	77.0 ± 0.8	74.7 ± 0.5	75.1 ± 0.9	0.873	1.13
8	Mal-acetyl	585.331	140.2 ± 5.0	59.9 ± 0.7	53.2 ± 0.7	61.2 ± 1.0	83.6 ± 1.5	76.9 ± 1.5	61.6 ± 0.7	0.671	0.932
9	Pet-*cis*-*p*-coumaroyl	625.170	121.3 ± 2.0	121.6 ± 0.3	119.2 ± 2.3	105.6 ± 3.2	93.6 ± 1.0	89.7 ± 0.1	81.8 ± 1.6	1.36	1.24
10	Peo-*trans*-*p*-coumaroyl	639.331	111.4 ± 1.3	110.6 ± 0.4	109.2 ± 1.2	90.6 ± 0.8	92.3 ± 1.6	77.1 ± 0.1	71.8 ± 0.3	1.23	1.08
11	Mal-*trans*-*p*-coumaroyl	639.331	2036.6 ± 1.5	2033.6 ± 0.3	1738.6 ± 2.4	1758.6 ± 1.6	1583.4 ± 2.4	1589.9 ± 0.2	1499.9 ± 1.9	22.8	22.7
	Total		7870.6	8922.4	7849.3	7222.0	7123.9	7142.8	6612.0	100	100

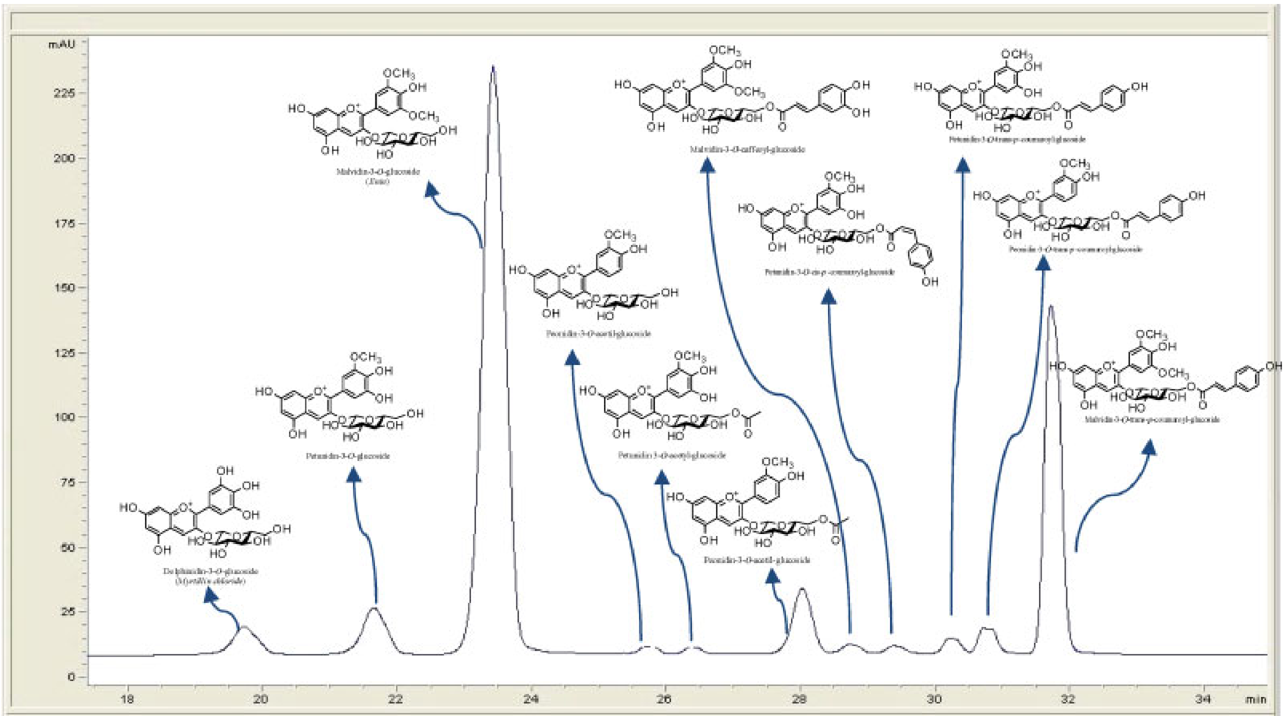


Figure 2. HPLC chromatogram of Aglianico grape skin extracts recorded after 30 min at 520 nm. The peak labels are those reported in Table 1.

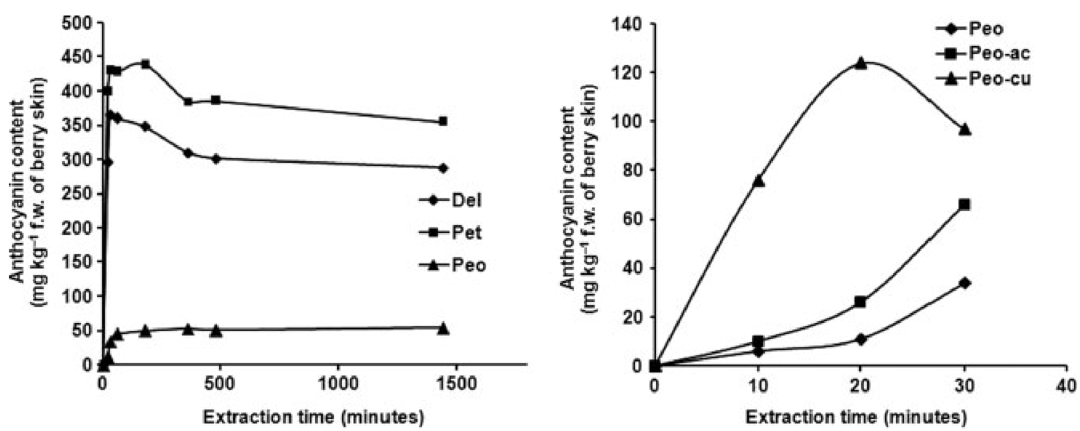


Figure 3. Levels of Del, Pet and Peo, and levels of Peo, Peo-acetyl and Peo-*trans-p*-coumaroyl detected at different extraction times ($n = 5$).

that it is more closely bonded to skin tissue, and needs more time to be released (Fig. 3). Among the acetyl derivatives, Peo-acetyl was transferred from skin more slowly than Pet-acetyl and Mal-acetyl, demonstrating that the lack of a hydroxy or methoxy group on the aglycon strongly affected compound extractability. Both Peo and Peo-acetyl showed a very similar trend, different to that of the coumaroyl derivative, which appeared to be more readily available (Fig. 3).

The anthocyanin profile of the wines was quite different from that of the grape skins (Table 3). The anthocyanin composition of the wine produced from Aglianico grapes shows that the total anthocyanin content was about one-tenth of the corresponding value found in berry skin (Table 3). This anthocyanin decrease can be considered normal for wine, as the greatest losses take place in the first months of ageing and are due to reactions during the fermentation process, condensation with tannins, breakdown by external factors (temperature, light, oxygen, etc.) and precipitation in colloidal colouring matter.[25,27] However, the total anthocyanin content of Aglianico wine is approximately twofold higher than Monastrell-J, Cabernet Sauvignon and Syrah, and approximately twofold higher than Monastrell-B and Merlot.[26]

The percentage content of Mal in the corresponding wine showed a slight decrease when compared to the value of berry skin (after a 24 h extraction), whereas Del values in berry skin and wine were similar (Tables 2 and 3). Mal was the predominant anthocyanin in Aglianico wine (50.95%), a situation common in several red wines, in which it forms the basis of their colour.[28–30] If compared to grapes, the relative amount of Pet in the wine decreased approximately sevenfold, whereas Peo increased approximately fourfold (Tables 2 and 3). As the polarity of Pet is higher than that of Peo and Mal, this compound is subjected to a strong absorption on yeast cell walls during fermentation,[27] and this could explain the observed decrease of Pet during winemaking. The high levels of Mal and Peo in Aglianico wine can

Table 3. Average anthocyanin composition (± standard deviation; $n = 11$) of 11 different samples of Aglianico wine

	Anthocyanin	Anthocyanin content (mg L^{-1})	(%)
1	Del	41.2 ± 0.4	6.11
2	Pet	27.2 ± 0.6	4.03
3	Mal	343.6 ± 1.9	50.95
4	Peo	20.7 ± 0.7	2.98
5	Pet-acetyl	20.4 ± 0.5	3.02
6	Peo-acetyl	55.2 ± 0.6	8.18
7	Mal-caffeoyl	0.0	0.00
8	Mal-acetyl	34.1 ± 0.8	5.06
9	Pet-*cis*-*p*-coumaroyl	29.7 ± 0.7	4.40
10	Peo-*trans*-*p*-coumaroyl	23.5 ± 1.1	3.48
11	Mal-*trans*-*p*-coumaroyl	78.7 ± 0.9	11.67
	Total	674.3	

be considered a positive factor, as these compounds participate in condensation reactions with quinonic species at a slower rate, so avoiding a fast wine oxidation, with a consequent loss of quality.[31]

The percentage contents of acetyl derivatives of Mal, Peo and Pet increased from 0.93%, 0.91% and 0.74% in berry skin to 5.05%, 3.02% and 8.18% in the wine, respectively, whereas the coumaroyl derivatives of Mal, Peo and Pet changed from 22.7%, 1.08% and 1.24% in berry skin to 11.68%, 3.48% and 4.40% in the wine, respectively (Tables 2 and 3). It appears that the ratio acetyl/coumaroyl anthocyanins of the wine (0.85) was sharply higher than the value in berry skin (0.10), indicating a strong presence of anthocyanin acetyl derivatives in the wine. Interestingly, Mal-caffeoyl detected in berry skin was not found in the wine (Table 3), even though very small amounts of this anthocyanin have been reported in some red wines.[12,27]

CONCLUSION

The results show that total anthocyanin contents in Aglianico berry skin and wine were higher than those found in other important red grape varieties subjected to culture conditions similar to those used in this work.[26,28–30] This demonstrates that anthocyanin fraction in Aglianico wine can be considered a major contributor to its total antioxidant capacity. In Aglianico wine, the concentration of the single anthocyanins did not always maintain the same relative content detected in berry skin. Finally, the observed enrichment of acetylated anthocyanins after vinification could ameliorate wine quality, as these compounds participate in intramolecular co-pigmentation processes, increasing the chromatic properties, ageing stability and product acceptance.[25,32] In the future, research into variations in the levels of anthocyanins and other polyphenols categories under various culture conditions may give a more complete picture of this grape variety and better define its health-promoting effects and organoleptic properties.

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