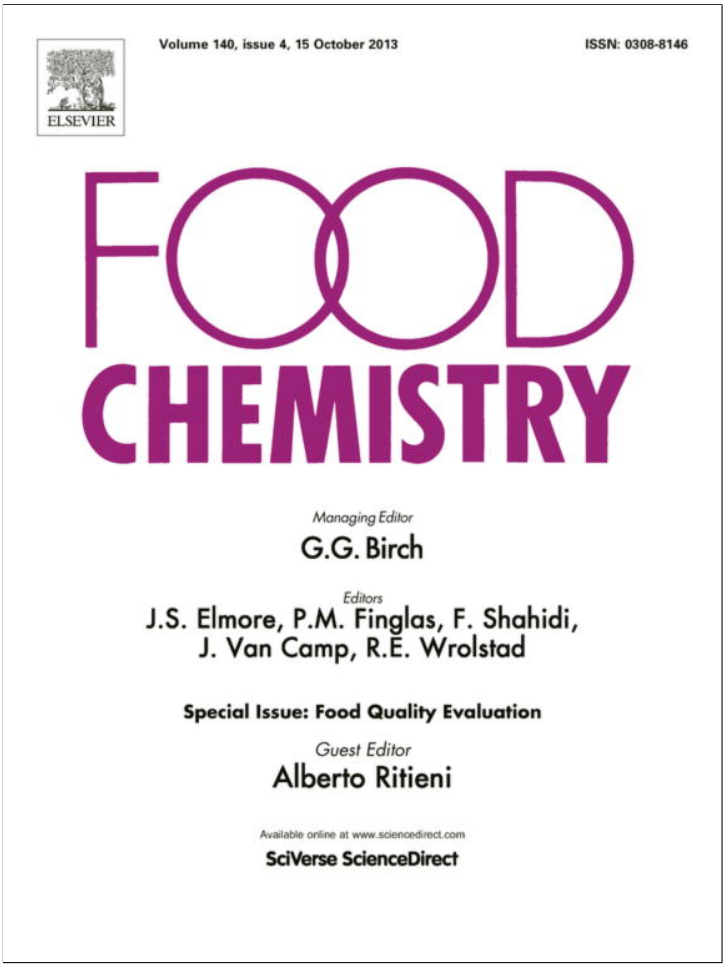

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Nutraceutical properties and polyphenolic profile of berry skin and wine of *Vitis vinifera* L. (cv. Aglianico)

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ABSTRACT

Red grapes are rich in phenolics, flavonoids, anthocyanins and resveratrol, all substances which have been suggested as having nutraceutical and health benefits. The berry skin and wine of grape cultivar *Vitis vinifera* L. (cv. Aglianico), grown in Basilicata (Southern Italy) were examined to determinate the presence of the above mentioned compounds as well as to establish the inorganic cation profile. HPLC analysis coupled with LC–ESI/MS/MS detected high contents of total flavonols and anthocyanins in berry skin and wine. The wine made with the same grape used for berry skin assays showed a notable presence of quercetin-3-*O*-glucoside (39.4% of total flavonols), and malvidin and petunidin derivatives (63.9% and 10.8% of total anthocyanins, respectively). The strong antioxidant ROS-scavenging activity, determined by both DPPH and FRAP assays, and the high resveratrol content confer high sensory characteristics resulted to be associated with positive nutraceutical properties of these grapes and wine. The level of *cis*-resveratrol was lower than *trans*-resveratrol in both berry skin and wine reaching 44.1 mg/kg and 0.3 mg/l, respectively. The cation profile presents low levels of Ca, Cu, K, Fe, Zn and Cd compared to numerous, important red wines, such as Monastrell and Tempranillo.

1. Introduction

An increasing body of experimental evidence has shown the health benefits of polyphenols, a large family of natural compounds widely distributed in dietary plants which need phenolic compounds for pigmentation, growth, reproduction, and for many other functions, such as radical scavenging, signalling and defence from pathogen and parasitic attack.

In grapes, four classes of flavonoids are commonly detected: flavonols, anthocyanins, flavan-3-ols and their polymeric forms, and condensed tannins (Clarke & Bakker, 2004, chap. 2). They are often leached out by grapes during the maceration period of wine-making and endow characteristics to grape varieties and wines. While anthocyanins are water-soluble pigments located in grape skins and seeds, which appear red, purple or blue, according to pH, flavonols are yellow pigments generally considered to act as UV protectants and free-radical scavengers (Downey, Dokoozlian, & Krstic, 2006). Resveratrol (3,5,4′-triidrossistilbene), the main stilbene synthesised in grape skin cells, is a non-flavonoid polyphenol that acts as a phytoalexin, being part of plant's defence system. Indeed, it is produced in plants in response to invading fungus, stress, injury, infection, or UV irradiation. Red wines contain high levels of resveratrol, as do grapes, raspberries, peanuts, and other plants (Goldberg & Soleas, 2003).

Wine flavonoids show beneficial effects on coronary heart disease, atherosclerosis, and some metabolic disorders, and they can inhibit carcinogenesis due to their antioxidant capacity (Vermerris & Nicholson, 2006, chap. 7). Generally, it has been established that an oxidation process is involved in the initial development steps of these disease, since an excess of reactive oxygen species (ROS) naturally formed during normal metabolism can damage biological macromolecules, such as proteins, lipids and nucleic acids (García-Alonso, Pascual-Teresa, Santos-Buelga, & Rivas-Gonzalo, 2004). Resveratrol has also been shown to reduce tumour incidence in animals by affecting one or more stages of cancer development. The strong antioxidant and radical-scavenging properties of resveratrol and flavonols, such as of quercetin, have been intensively

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studied in both grapes and wines (Davalós & Lasunción, 2009). This wide range of health-promoting compounds suggests that several different and interrelated mechanisms of action are involved in the enhancement of the total antioxidant effects of the polyphenol family present in grape and wine.

The study of the components present in wine and in grapes, as flavonols and anthocyanins, may also contribute to winegrape taxonomic characterisation and for certifying wine quality and origin (Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 2006).

Grape and wine ionome, which describes the content of all mineral nutrients and trace elements, is today a poorly studied sector. The minerals, taken up by the grape and wine from the soil usually make up approximately 0.2–0.6% of the fresh weight of the fruit (Ribéreau-Gayon et al., 2006, chap. 4). K mineral cations, including, Na and Fe, are essential to the human organism and, together with Ca, Co, Cu, Fe, Mn, Se, Zn, play a crucial role for their nutraceutical properties. Consumption of wine in moderate quantities may significantly cover metal physiological needs. Numerous of these inorganic cations, naturally present in must and wine at non-toxic concentrations (e.g., K, Fe and Cu), play a major role both in winemaking and wine quality (Ribéreau-Gayon et al., 2006, chap. 4). Heavy metals as Pb, Hg and Cd, naturally present as sulphides in trace concentrations in the fruit, usually precipitate during fermentation, and their presence is important for grape and wine toxicology purposes.

On these bases, the present paper aims at investigating the presence of the above cited compounds in the berry skins and wine of Aglianico (*Vitis vinifera* L.), one of the most ancient grape cultivar, introduced by Greeks into Southern Italy in pre-Roman times, and to estimate their biological properties.

2. Materials and methods

2.1. Reagents and standards

All chemicals and reagents were analytical-reagent at HPLC grade. DPPH· (1,1-diphenyl-2-picrilhydrazyl), 2,4,6-tris-2,4, 6-tripiridyl-2-triazine (TPTZ), iron (III) chloride (dry), 6-hydroxy-2,5, 7,8-tetramethylchroman-2-carboxylic acid (Trolox), (+)-catechin hydrate, gallic acid monohydrate, aluminium chloride (dry), malvin (malvidin-3-*O*-glucoside) chloride, resveratrol (*cis*/*trans* isomers), Folin & Ciocalteu's phenol reagent, were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Methyl alcohol (RPE) was purchased from Carlo Erba (Milano, Italy). HNO_3 (Suprapur grade) and multielemental standard stock solutions (1000 mg/l) were purchased from Merck (Darmstadt, Germany).

2.2. 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, this production area falls within climatic region 5, *very hot*. During the experiment, the temperatures ranged between 0 and 38.5 °C, while cumulative rainfall of the period was 245 mm. The experimental plot, of about 0.30 ha, consisted of ten rows of spur-pruned vines to a permanent horizontal unilateral cordon. Each vine, decked at 0.60 m above the ground, was characterised by about 8 spurs of 2 to 3 buds each. The distance between the vines was of 2.5 × 1.0 m, with a final plant density of 4000 vines ha^{-1}. Rows were north–south oriented. The rows were oriented in a north–south direction. The soil was classified as a clay–loam.

The plants were irrigated weekly from 9 June to 1 August (from fruit set to veraison) using a water amount equal to 100% of cultural evapotranspiration (ETc). The value of ETc was calculated using ETo × Kc, where ETo is the reference evapotranspiration calculated according to Hargreaves method, and Kc is the cultural coefficient during the experimental period, equal to 0.6 for grapevine, according to Allen, Pereira, Raes, and Smith (1998). The seasonal irrigation volume was of 960 m^3 ha^{-1} (240 L $plant^{-1}$). Each vine was irrigated by two drip emitter per plant discharging 4 L h^{-1} each.

2.3. Flavonol and anthocyanin extractions from berry skin and wine of Aglianico grapes

At harvest, on 27 September 2008, three clusters per plant were randomly sampled in the central and well-irradiated area of the canopy of five plants ($n = 5$) located in the central part of the row, where microclimatic conditions and soil physic-chemical characteristics were similar. From the clusters of each plant ($n = 5$), the berries were immediately detached, weighted and pooled together, and immediately stored at 4 °C in sterile plastic bags. Immediately after the transportation in the laboratory, the berries with a weight between 0.60 and 1.25 g (the most abundant and representative weight class) were rapidly stored at −80 °C in sterile polyethylene containers before the following analyses. Successively, frozen berries were peeled with a scalpel and the skins collected. This operation was carried out in an efficient way, as at low temperature the skin is easily removed by the rest of the berry.

From each sample ($n = 5$), five grams of berry skin were placed in a 100 ml methanol–HCl 0.75% (w/w) solution at room temperature. The extraction was monitored for 24 h. Furthermore, the wine coming from nine chosen plants located in the central part of the row (nine samples of one-year-old wine; $n = 9$) was bottled in glass bottles previously purged with gaseous nitrogen. The bottles were closed with corks and stored in the dark at 15 °C until analyses.

2.4. Identification and quantification of flavonols and anthocyanins

Separation of the flavonols and anthocyanins from berry skin and wine was performed by high performance liquid chromatography (HPLC) and the analysis of crude extracts was performed after filtration with nylon filters of 0.45 mm (Teknokroma, Barcelona, Spain) to remove any solid residue. The structural identification was carried out by comparison of UV and retention times of our samples with authentic commercial samples according to a methodology reported in a previous paper (Downey et al., 2006). These data were validated by the LC–ESI/MS/MS analysis.

The HPLC analysis were carried out by a Finnigan HPLC system (Thermo Electron Corporation, San Jose, CA, USA), a photodiode array detector (DAD). For detection of compounds, the chromatograms were recorded at 260, 320 and 520 nm in the photodiode detector. The different phenol compounds analysed were tentatively identified according to their order of elution, retention times of standard pure compounds, characteristics of the UV–Vis or fluorescence spectra, and by comparison with a bibliographic data. A complete UV–Vis spectrum database of all extracts and wine components was build and used to assess peak identification. Elution conditions consisted in 10% formic acid in water (Solvent A) and 10% formic acid in methanol (Solvent B) gradient at a flow rate of 1.0 ml/min. The column used was a C-18 Zorbax (150 mm × 4.6 mm, 5 μm packing; Agilent, USA) protected by an Agilent C-18 guard column. The elution conditions were: 0 min, 18% B; 14 min, 29% B; 16 min, 32% B; 18 min, 41% B; 18.1 min, 30% B; 29 min, 41% B; 32 min, 50% B; 34.5 min, 100% B; 35–38 min, 18% B. Calibration curves consisted in 0.001–1 mg/ml catechin and 0.05–1 mg/ml malvidin-3-glucoside standard solutions.

The identification of anthocyanins and flavonols was confirmed by a liquid chromatography electrospray ionisation tandem mass spectrometry (LC–ESI/MS/MS) analysis using a HP1100 HPLC

system (Agilent Technologies Inc., CA, USA) coupled to PE-Sciex API-2000 triple-quadrupole mass spectrometer (Warrington, Cheshire, UK) equipped with a Turbospray (TSI) source. MS detection was carried out in positive ion mode for anthocyanins and negative ion mode for flavonols at unit resolution using a mass range of 150–1500 m/z and a mass peak width of 0.7 ± 0.1. Selected ion monitoring (SIM) experiments were carried out using the following operational parameters: vaporiser, 350 °C; heated capillary, 150–200 °C; carrier gas, nitrogen, at a sheath pressure of 70 psi; auxiliary gas, nitrogen, to assist in nebulisation, at a pressure of 30 psi; de-clustering potential, 44.0 eV; focusing potential, 340.0 eV; entrance potential, 10.0 eV; collision energy, 33.0 eV for ion decomposition in the collision cell at 0.8 mTorr.

2.5. Determination of cis- and trans-resveratrol

The quantification of *cis*- and *trans*-resveratrol in skin extracts and wine samples was carried out on the Finnigan HPLC equipment reported above. Separation was achieved using a Zorbax C-8 column (150 × 4.6 mm, 5 μm packing; Agilent, USA) and a mobile phase of 0.1% aqueous formic acid (solvent A) and acetonitrile (solvent B) delivered in isocratic elution mode at 25% B (v/v) at a flow rate of 1 ml/min. Calibration curves were plotted from 0.005 to 10 mg/ml. Wine samples (20 ml) were directly injected after filtration through a 0.45 mm membrane filter. A photodiode array detector was used, and quantification was done at 285 nm for *cis*- and *trans*-resveratrol.

2.6. Total antioxidant capacity

For each antioxidant assay, a Trolox aliquot was used to develop a 50–500 μmol/l standard curve. All data were expressed as Trolox Equivalents (μmol TE/100 ml wine). Spectrophotometric analyses were performed using a Jasco V-530 UV–vis spectrophotometer (Tokyo, Japan) set at appropriate wavelengths to each assay. The ability of the berry skin extracts and the wine samples to scavenge the DPPH radical was measured according to Brand-Williams, Cuvelier, & Berset (1995). Aliquots (20 μL) of berry skin extract or of wine were added to 3 ml of DPPH solution (6×10^{-5} mol/l) and the absorbance was determined at λ 515 nm every 5 min until the steady state. The anti-oxidant potential of berry skin extracts and wines was also determined using a FRAP assay, Ferric Reducing Ability of Plasma, as a measure of antioxidant power. A solution of 10 mmol/l TPTZ in 40 mmol/l HCl and 12 mmol/l $FeCl_2$ was diluted in 300 mmol/l sodium acetate buffer (pH 3.6) at a ratio of 1:1:10. Aliquots (20 μL) of skin extract or wine were added to 3 mL of the FRAP solution and the absorbance was determined at 593 nm every 5 min until the steady state was reached.

2.7. Metal determination

Determination of the main metals in both berry skin extracts and wine samples was performed by quadrupole based inductively coupled plasma mass spectrometry, ICP-QMS (Elan DRC II, Perkin-Elmer SCIEX, CT, USA). Operational parameters were the following: sample uptake rate, 1 ml/min; sample introduction, Meinhard nebuliser with cyclonic spray chamber; gas flow rates (L min^{-1}): plasma, 15; auxiliary, 1.0; nebuliser, 0.85; dwell time, 50 ms; No. of replicate, 5; interface, Pt cones; extraction lens voltage, optimised for maximum I (^{56}Fe). High purity He (99.9999% He) and H_2 (99.9995% H_2) were used, in order to minimise the potential problems caused by unidentified reactive contaminant species in the cell. The high radio frequency power (1500 W) helped to maintain stable plasma in the presence of ethanol. Before use, all glassware and plastic containers were cleaned by washing with 10% ultra-pure grade HNO_3 for at least 24 h, and then rinsed with copious quantities of ultra-pure water obtained with a Milli-Q purification system (Millipore Inc., Bedford, MA, USA). The wine samples were collected from the glass bottles by cautiously removing the corks, conditioning the necks by 5% HNO_3, and then aspirating the liquid with no contaminating pipettes. The plasma instability, related to the ethanol content in wine (Taylor, Longerich, & Greenough, 2003), were minimised by a simple 1:5 dilution with 1% HNO_3. A 2.5% ethanol matrix for standards and blanks was used to approximate the 1:5 diluted wine matrixes. The calibration solutions were prepared from multi-elemental standard stock solutions of 1000 mg/l. The calibration curves were obtained by using at least 6 calibration solutions. Reagent blanks containing ultra-pure water were additionally analysed in order to control the purity of reagents and laboratory equipment. Standards and blanks were subjected to the same treatment as the wine samples.

2.8. Statistics

Unless otherwise stated, all of the experimental results were expressed as mean ± standard deviation of measurements from independent samples ($n = 5$ for berry skin and $n = 9$ for wine). A one-way ANOVA was performed on the means to determine whether they differed significantly. Significant differences were determined at $P \leqslant 0.05$, according to Fisher's LSD test.

3. Results and discussion

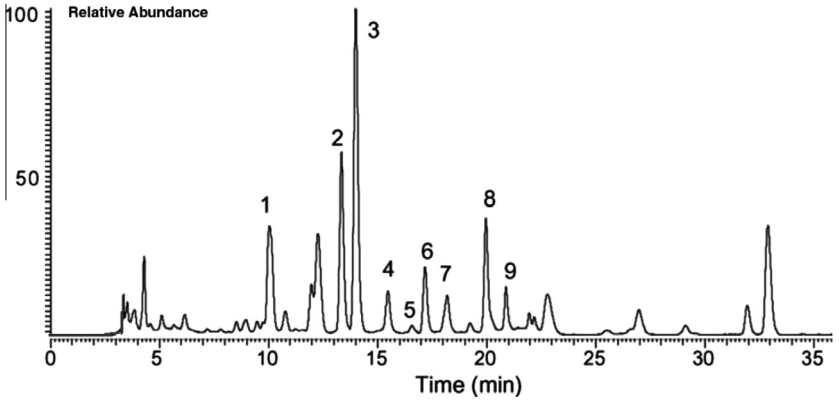
3.1. Flavonols and anthocyanins

The flavonols present in berry skin and in corresponding wine of Aglianico grapes, their HPLC and mass spectrometry data are reported in Tables 1 and 2, respectively. The flavonol content of berry skins (1036.7 mg/kg fresh weight) was 15-fold higher than in the corresponding wine (69.5 mg/l) Among the nine flavonols detected in Aglianico grape and wine, quercetin-3-*O*-glucoside and, to a lesser extent, quercetin-3-*O*-glucuronide, were the most abundant flavonols both in berry skin and wine, in agreement with previous works (Gawel, 1998). These compounds are involved in the long-term colour stability of red wines and in the improvement of organoleptic properties. Moreover, they are associated to numerous positive nutraceutical properties and health benefits (Gawel, 1998). Myricetin-3-*O*-glucoside, largely present in berry skin, decreased in wine, indicating a lowering of its bioavailability during the winemaking process. Generally, wine samples have shown an important peak corresponding to free myricetin suggesting that this flavonol seems to be easily hydrolysed (Castillo-Muñoz, Gómez-Alonso, García-Romero, & Hermosín-Gutiérrez, 2007). Isorhamnetin-3-*O*-glucoside and kaempferol-3-*O*-caffeoylate reverse the common flavonol content by doubling their presence in the wine when compared to the berry skin. Isorhamnetin inhibits adipogenesis through down-regulation of PPAR-gamma and C/EBP-alpha in 3T3-L1 Cells (Lee, Jung, & Lee, 2009). Kaempferol seems to prevent arteriosclerosis by inhibiting the oxidation of low density lipoprotein and the formation of platelets in the blood. Current evidence indicates that kaempferol not only protects LDL from oxidation but also prevents atherogenesis through suppressing macrophage uptake of oxLDL. Numerous studies showed kaempferol may have health benefits for people at risk of cancer (Zhang, Chen, Li, Chen, & Yao, 2008). Wine content of laricitrin-3-*O*-rhamnose, laricitrin-3-*O*-galactoside, syringetin-3-*O*-galactoside and kaempferol-3-*O*-glucoside retain proportionality with the total flavonoid content in berry skin. During the ripening of the grapes, flavonols are accumulated in the berry skin and their absolute and relative content can be influenced by many abiotic factors, including water availability and temperature (Downey

Table 1
Flavonol content (±standard deviation) of berry skin ($n = 5$) and wine ($n = 9$) of Aglianico grapes. The percent values followed by an asterisk were statistically different between berry skin and wine ($P \leqslant 0.05$).

Compound	Berry skin		Wine	
	(mg/kg fresh weight)	%	(mg/l)	%
Myricetin-3-*O*-glucoside	142.1 ± 1.2	13.7	7.70 ± 0.9	11.1
Quercetin-3-*O*-glucuronide	170.6 ± 1.1	16.5	9.07 ± 0.8	13.1*
Quercetin-3-*O*-glucoside	441.3 ± 7.4	42.6	27.39 ± 1.5	39.4*
Laricitrin-3-*O*-galactoside	38.7 ± 0.9	3.7	2.14 ± 0.8	3.1
Kaempferol-3-*O*-glucoside	27.2 ± 1.1	2.6	1.40 ± 0.7	2.0
Laricitrin-3-*O*-rhamnose-7-*O*-trihydroxycinnamic acid	56.1 ± 0.7	5.4	3.90 ± 0.3	5.6
Kaempferol-3-*O*-caffeoylate	46.5 ± 0.9	4.5	5.44 ± 0.6	7.8*
Isorhamnetin-3-*O*-glucoside	67.0 ± 0.8	6.5	8.69 ± 0.5	12.5*
Syringetin-3-*O*-galactoside	47.2 ± 0.4	4.6	3.74 ± 0.4	5.4
Total	1036.7		69.47	

Table 2
Mass chromatogram and mass spectrometry data of the flavonols detected in Aglianico berry skin and wine.



Peak	Compound	*m/z* $(M-H)^-$	MS^{2a}	MS^{3a}	MS^{4a}
1	Myricetin-3-*O*-glucoside	479	316/317	242, 270/271, 287	171, 199, 227
2	Quercetin-3-*O*-glucuronide	477	301	151, 179,193, 257, 273	151
3	Quercetin-3-*O*-glucoside	463	301	151, 179,193, 257, 273	151
4	Laricitrin-3-*O*-galactoside	493	330, 331	151, 179,193, 316, 317	151, 164, 179, 219, 244, 270/271,287/288
5	Kaempferol-3-*O*-glucoside	447	255, 284/285, 327, 401, 419, 429	227, 239, 255/256	212, 227
6	Laricitrin-3-*O*-rhamnose-7-*O*-trihydroxycinnamic acid	655	303, 314, 329, 347, 475, 501, 509	314	299
7	Kaempferol-3-*O*-caffeoylate	447	284/285	227, 239, 255/256	212, 227
8	Isorhamnetin-3-*O*-glucoside	477	271, 285, 314/315, 357	243, 257, 271, 285/286, 299/300	241/270
9	Syringetin-3-*O*-galactoside	507	344/345, 387, 479, 489	330	

[a] Base peak (100%) is underlined.

et al., 2006). Considering that water supplies in this experiment were not a limiting factor, we compared the levels of flavonols and anthocyanins with those found in grapevines grown under similar cultivation and environmental conditions. From this comparison, it appears that total quercetin content detected in Aglianico berry skin (441.3 and 170.6 mg/kg for quercetin-3-*O*-glucuronide and quercetin-3-*O*-glucoside, respectively), as well as the levels of other flavonols detected, are higher than those found in some of the most important red grape varieties (Makris, Kallithraka, & Kefalas, 2006). The same difference was found considering wines, where Aglianico ranks at the first places for quercetin derivatives and total flavonols, with levels comparable to wines produced from high-quality red wine varieties such as Cabernet Sauvignon, Sangiovese, Primitivo, Merlot, and Zinfandel (Makris et al., 2006).

The anthocyanin levels and the mass spectrometry parameters of the berry skin and wine samples are shown in Tables 3 and 4, respectively. Among the fruits and vegetables commonly consumed, grapes and their associated products are regarded as the most important source of our dietary anthocyanins. These compounds have been shown to contribute to the strong protection of the red grape and wine against low-density lipoprotein oxidation (Frankel, Bosanek, Meyer, Silliman, & Kirk, 1998). Recent studies have demonstrated that the long-term intake of anthocyanins,

Table 3
Anthocyanin content (±standard deviation) of the berry skin ($n = 5$) and wine ($n = 9$) of Aglianico grapes. ND = not detected. The percent values followed by an asterisk were statistically different between berry skin and wine ($P \leqslant 0.05$).

Compound	Berry skin		Wine	
	(mg/kg fresh weight)	%	(mg/l)	%
Delphinidin-3-*O*-glucoside	369.5 ± 6.2	3.7	40.8 ± 1.6	5.7
Cyanidin-3-*O*-glucoside	353.8 ± 3.2	3.5	37.3 ± 0.9	5.2
Petunidin-3-*O*-glucoside	504.6 ± 1.1	5.0	27.7 ± 1.2	3.9
Peonidin-3-*O*-glucoside	33.5 ± 0.9	0.3	21.2 ± 0.6	3.0*
Malvidin-3-*O*-glucoside	5613.7 ± 9.1	56.2	344.2 ± 1.5	48.1*
Delphinidin-3-*O*-acetyl-glucoside	222.5 ± 2.1	2.2	2.1 ± 0.8	0.3
Cyanidin-3-*O*-acetyl-glucoside	173.2 ± 1.2	1.7	0.9 ± 0.7	0.1
Petunidin-3-*O*-acetyl-glucoside	84.4 ± 0.7	0.8	19.9 ± 0.9	2.8
Peonidin-3-*O*-acetyl-glucoside	140.7 ± 2.2	1.4	54.6 ± 1.2	7.6*
Petunidin-(6-*O*-caffeoyl)-glucoside	34.2 ± 0.6	0.3	ND	ND
Malvidin-3-*O*-acetyl-glucoside	57.0 ± 0.8	0.6	34.6 ± 0.7	4.8*
Malvidin-(6-*O*-caffeoyl)-glucoside	112.6 ± 1.9	1.1	0.7 ± 0.2	0.1
Cyanidin-3-(6-*O*-coumaroyl)-glucoside (*trans* isomer)	63.8 ± 0.8	0.6	0.4 ± 0.1	0.0
Petunidin-3-(6-*O*-coumaroyl)-glucoside (*trans* isomer)	140.9 ± 0.7	1.4	29.2 ± 1.2	4.1*
Peonidin-3-(6-*O*-coumaroyl)-glucoside (*trans* isomer)	106.5 ± 1.2	1.1	24.6 ± 0.9	3.4*
Malvidin-3-(6-*O*-coumaroyl)-glucoside (*trans* isomer)	1985.2 ± 8.5	19.9	78.1 ± 0.8	10.9*
Total	9996.1		716.3	

Table 4
Mass spectrometry data of anthocyanins detected in Aglianico berry skin and wine.

Peak	Compound	m/z $(M+H)^+$	MS[2a]	MS[3a]	MS[4a]
1	Delphinidin-3-*O*-glucoside	465	303	229, 257, 303	229, 257
2	Cyanidin-3-*O*-glucoside	449	287	213, 231, 241, 259, 287	213, 231, 241, 259, 287
3	Petunidin-3-*O*-glucoside	479	317	257, 274, 302, 317	218, 228, 246, 274
4	Peonidin-3-*O*-glucoside	463	301	286	230, 258, 268
5	Malvidin-3-*O*-glucoside	493	331	179, 242, 270, 287, 299, 315/316	213, 257, 285, 287, 313, 315
6	Delphinidin-3-*O*-acetyl-glucoside	507	303	229, 257, 303	229, 257, 303
7	Cyanidin-3-*O*-acetyl-glucoside	491	287	213, 231, 259, 287	213, 231, 259, 287
8	Petunidin-3-*O*- acetyl-glucoside	521	302, 317	218, 228, 246, 256, 274	135, 149, 153, 163, 181
9	Peonidin-3-*O*- acetyl-glucoside	505	301	286	230, 258, 268
10	Petunidin-(6-*O*-caffeoyl)-glucoside	641	317	302	218, 228, 246, 274
11	Malvidin-3-*O*-acetyl-glucoside	535	331	179, 242, 270, 299, 315, 331	257, 285, 287, 313, 315
12	Malvidin-(6-*O*-caffeoyl)-glucoside	655	331	179, 242, 270, 287, 299, 315/316, 331	257, 285, 287, 313, 315
13	Cyanidin-3-(6-*O*-coumaroyl)-glucoside (*trans* isomer)	595	287	213, 231, 259, 287	213, 231, 259, 287
14	Petunidin-3-(6-*O*-coumaroyl)-glucoside (*trans* isomer)	625	317	274, 302	218, 228, 246, 274
15	Peonidin-3-(6-*O*-coumaroyl)-glucoside (*trans* isomer)	609	301	286	230, 258, 268
16	Malvidin-3-(6-*O*-coumaroyl)-glucoside (*trans* isomer)	639	331	179, 242, 270, 287, 299, 315/316, 331	225, 253, 281, 299

[a] Base peak (100%) is underlined.

which were administered as food matrix or enriched fractions, changed the markers for the oxidative status in some tissues and affected antioxidant enzyme expression levels and activities when compared with animals that did not receive polyphenols in the diet (Hassimotto & Lajolo, 2011). Thus, considering the dietary intake of anthocyanins (approximately 100 mg/die) and their potential health benefits, the grape and wine samples could be regarded as a valuable anthocyanin source suitable for use as dietary supplement. Malvidin-3-*O*-glucoside was found to be the main anthocyanin present along with its coumaroyl derivative, accounting for 87% and 59% of total content in berry skin and wine, respectively (Table 3). Besides the malvidin-3-*O*-glucoside and *trans* malvidin-3-(6-*O*-coumaroyl)-glucoside, differences between anthocyanin present in berry skin and wine were found for peonidin derivatives, malvidin-3-*O*-acetyl-glucoside and *trans* petunidin-3-(6-*O*-coumaroyl)-glucoside, with percentages significantly higher in wine than in berry skin (Table 3). Total anthocyanin content in berry skin was approximately 14-fold of the corresponding value found in wine (9996.1 mg/kg and 716.3 mg/l, respectively), a proportion not differing greatly from that found in Spanish variety Jumilla-Monastrell (Romero-Cascales, Ortega-Regules, López-Roca, Fernández-Fernández, & Gómez-Plaza, 2005).

Generally, Aglianico wine appeared to have a high anthocyanin content (716.3 mg/l) in comparison with the profiles of other high-quality red wines (Gómez-Alonso, Fernández-González, Mena, Martínez, & García-Romero, 2007). Furthermore, the concentration of malvidin and petunidin derivatives (63.9% and 10.8% of total anthocyanins, respectively), in Aglianico wine, is comparable with their presence in other well-known red wines, such as Tempranillo (Revilla, García-Beneytez, & Cabello, 2009), Cabernet Sauvignon (Romero-Cascales et al., 2005), Monastrell-J (Romero-Cascales et al., 2005), Shiraz (Romero-Cascales et al., 2005), and Pinot Noir and Muscat Rouge (Boss, Davies, & Robinson, 1996). In particular, the high levels of acetylated anthocyanins detected in Aglianico wine (e.g., peonidin-3-*O*-acetylglucoside and petunidin-3-*O*-acetylglucoside), represent another positive sensory parameter as they confer a deep red colour and organoleptic attributes (Santos-Buelga & de Freitas, 2009).

3.2. Resveratrol and total antioxidant capacity

In agreement with previous works (Feijòo, Moreno, & Falque, 2008), the content of *trans*-resveratrol in wine was significantly higher than that of *cis*-resveratrol (Table 5) and lower in wine than

Table 5
trans- and *cis*-Resveratrol content (±standard deviation) of the berry skin ($n = 5$) and wine ($n = 9$) from Aglianico grapes.

Samples	Berry skin (mg/kg fresh weight)	Wine (mg/l)
trans-Resveratrol	441.41 ± 2.1	3.79 ± 0.9
cis-Resveratrol	163.63 ± 1.6	2.09 ± 1.1

Table 6
Total antioxidant capacity expressed as Trolox of the extracts from the berry skin ($n = 5$) and wine ($n = 9$) of Aglianico grapes determined by DPPH· and FRAP assays at the steady state (DPPH·, 45 min; FRAP, 55 min).

Sample	Assay method	
	DPPH· (μmol/l)	FRAP (μmol/l)
Berry skin	443.6	1095.7
Wine	1550.0	2200.0

Table 7
Macro- and micro-element content of berry skin ($n = 5$) and wine ($n = 9$) from Aglianico grapes. ND = not detected.

Element	Berry skin (mg/kg dry weight)	Wine (μg/l)
Ag	0.22 ± 0.04	2.16 ± 0.95
Al	8.10 ± 0.94	176.51 ± 0.02
Ca	332.99 ± 1.12	8.34 ± 0.15[a]
Cd	0.02 ± 0.06	0.20 ± 0.01
Co	0.51 ± 0.07	9.42 ± 0.85
Cu	23.82 ± 0.79	1.60 ± 0.90[a]
Fe	28.59 ± 0.73	190.51 ± 0.91
Ga	0.15 ± 0.05	9.35 ± 1.05
K	62.40 ± 0.21	691.00 ± 0.05
Mg	60.40 ± 2.53	6.12 ± 0.03
Mn	0.81 ± 0.03	17.34 ± 0.22
Mo	0.77 ± 0.02	17.75 ± 0.85
Na	1.16 ± 0.91	9.31 ± 0.20
Pb	1.76 ± 0.09	61.96 ± 0.96
Pt	0.01 ± 0.002	0.32 ± 0.04
Ru	3.67 ± 0.91	50.71 ± 1.23
Se	0.58 ± 0.09	ND
Sn	1.27 ± 0.75	16.27 ± 1.12
V	1.74 ± 0.87	29.94 ± 1.12
Zn	47.11 ± 1.11	4.55 ± 0.95

[a] Concentration in mg/l.

in berry skin because of the scarce stability of stilbenes during winemaking processes (Stervbo, Vang, & Bonnesen, 2007). The wine total resveratrol presence (5.88 mg/l) seen in Table 5 was higher than that found in many red wines usually assessed as 1.9 ± 1.7 mg/l (Stervbo et al., 2007) and comparable to the level found in some wines, Pinot Nero and Merlot, famous for the high concentration of the *trans* isomer (Stervbo et al., 2007).

The results show that resveratrol was present in Aglianico wine at higher concentrations than in the common red wines. This compound has a variety of bioactivity related to its antioxidant properties, such as cardioprotective, anti-cancer, anti-inflammation, anti-ageing and anti-microbial activities. Moreover, some studies supported Sinclair's hypothesis that the effects of resveratrol are indeed due to the activation of the Sirtuin 1 gene which is involved in life extension (Wood et al., 2004).

Owing to the complex reactivity of phytochemicals, the antioxidant activities of food and food extracts cannot be evaluated by only a single method, but at least two test systems have been recommended for the determination of antioxidant activity to establish authenticity (Schlesier, Harwat, Bohm, & Bitsch, 2002). For this reason, the antioxidant activity of wines was determined by two spectrophotometric methods, DPPH and FRAP tests, and expressed as trolox equivalents (TEs). The reduction of DPPH absorption is indicative of the capacity of the samples to scavenge free radicals, while the FRAP method is used to determine the capacity of reductants in a sample. The total antioxidant capacity, evaluated by FRAP and DPPH tests, was higher for wine than for berry skin exctracts (about 2- to 4-fold) (Table 6), due to the increased presence of malvidin, peonidin, cyanidin, delphidin and petunidin derivatives in wine (Radovanovi, Radovanovi, & Jovanievi, 2009).

3.3. Metals

Excess of Fe and Cu cations in wine, determines turbidity and causes significant instability owing to catalysing oxidative reactions which modify taste characteristics. Furthermore, wine haziness induction (commonly called 'casse') is due to unstable colloid formation arising from reaction between Fe, Cu, proteins and other wine components. For all these reasons, a high level of these minerals is undesirable as is the presence of poorly soluble Ca which contributes to colloid flocculation and salt precipitation. If compared to the mean values of Fe, Cu and Ca detected in a broad range of Southern Italian red wines (Volpe et al., 2009) and American red wines (Bentlin, Pulgati, Dressler, & Pozebon, 2011), the Aglianico wine samples presented low levels of these three elements (190.51, 1.60 and 8.34 mg/l for Fe, Cu and Ca, respectively) (Table 7). While the mean value of K concentration in wine is approximately 1 mg/l (Conde et al., 2007), Aglianico wine samples resulted to have a mean concentration of 0.691 mg/l (Table 7). This is a positive feature, as a high K level negatively affects wine quality, colour, stability and taste, depending on potassium bitartrate formation (Conde et al., 2007). Some of the heavy metals present into the wine, such as Cd, Pb and Zn may be derived from soil contaminants, fungicidal residues, or winery equipments, and could represent a danger for human health if present in high concentrations. In our wine samples, the levels of Cd and Zn (0.20 and 4.55 μg/l, respectively) were markedly low if compared to the levels usually detected in red wines (Volpe et al., 2009). The Pb concentration (61.96 μg/l), under the limit fixed by the Organization Internationale de la Vigne et du Vin (150 μg/l; O.I.V., 2011) is not negligible. Among heavy metals, Mn was present at a low level (17.34 μg/l) in wine (Table 7), and this could be related to its greater presence in grape seeds than in berry skin (Conde et al., 2007).

Finally, the optimal balance of macro- and micro-elements in Aglianico grape and wine can give a definitive contribution to defining the organoleptic and nutraceutical profile of this important but poorly studied grape variety.

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