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# Biochemical features of native red wines and genetic diversity of the corresponding grape varieties from Campania region

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ABSTRACT

Campania region has always been considered one of the most appreciated Italian districts for wine production. Wine distinctiveness arises from their native grapevines. To better define the chemical profile of Campania autochthonous red grape varieties, we analysed the phenolic composition of Aglianico di Taurasi, Aglianico del Vulture, Aglianico del Taburno, Piedirosso wines, and a minor native variety, Lingua di Femmina in comparison with Merlot and Cabernet Sauvignon, as reference cultivars. A genetic profiling was also carried out using microsatellite molecular markers with high polymorphic and unambiguous profiles. Principal component analysis applied to 72 wines based on the 18 biochemical parameters, explained 77.6% of the total variance and highlighted important biological entities providing insightful patterns. Moreover, comparison of SSR-based data with phenylpropanoid molecules exhibited a statistically significant correlation. Our approach might be reasonably adopted for future characterisations and traceability of grapevines and corresponding wines.



## 1. Introduction

Grapes (*Vitis vinifera* L.) belong to the world's largest fruit crops, as they cover a total area of 7.6 million hectares, with a global production of around 68 million tonnes in 2010 (International Organisation of Vine, 2012). The greatest proportion of grapes is employed for wine production, likely the most important use of grapes in terms of tradition, literature and even religion. According to OIV Statistical Report on World Vitiviniculture (International Organisation of Vine & Wine, 2012), in 2010 about 265 million of hectoliters of wine were produced worldwide, 20 million of which only in Europe, with Italy (48.5 Mhl), France (45.7 Mhl), and Spain (35.2 Mhl) as the world's leading wine-producing countries. In Italy, grapevines cover an area of about 800,000 ha, unevenly distributed all over the country. Campania region, due to its particular climatic conditions and fertility of its soil, is historically considered one of the most appreciated Italian districts for wine production. Campania wine distinctivenesses arise from the many autochthonous red grape varieties, such as Aglianico biotypes ('Aglianico di Taurasi', A. del Vulture' and 'A. del Taburno') and 'Piedirosso'. In addition, a series of minor, still not-well characterised, grape varieties is gaining an increasing interest. Given the renewed quality of their wines, it appears of particular relevance for the wine industry and marketing to preserve the valuable traits linked to genetic constitution, geographical origin of production and unique vinification technologies. In addition, the European Union is particularly interested in agricultural productions that combine safety and quality attributes with a clear regional identity (EC regulations 2081/92 and 1898/06). Attention to food authentication is due to several reasons, including health, media attention, specific organoleptic qualities of regional products (Luykx, Peters, van Ruth, & Bouwmeester, 2008).

In this scenario, the efficient assessment of food products authenticity is a major challenge for both producers and consumers. Organic and inorganic wine constituents (Kment et al., 2005), as well as wine sensory attributes, are usually used to distinguish wines according to vinification technology, area of origin and variety. Polyphenols are a class of grape organic compounds that confer wine important sensory properties as appearance, taste and mouthfeel. In particular, anthocyanins are mainly responsible for colour and proanthocyanins for bitterness and astringency (Arnold, Noble, & Singleton, 1980). In addition, many polyphenolic compounds display significant benefits to human nutrition and health (Bozan, Tosun, & Özcan, 2008). According to Ribéreau-Gayon (1982), the phenolic fingerprint is typical of each individual cultivar so that the analysis of anthocyanins and flavonoids has been used to distinguish grape varieties (Berente, García,

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Reichenbächer, & Danzer, 2000). Anthocyanin composition, in particular, is quite distinctive and its determination can be a parameter to assess grape authenticity (Revilla, Garcia-Beneytez, Cabello, Martin-Ortega, & Ryan, 2001). Due to the importance of polyphenols in determining the overall grape and grape-derived products qualities as well as in differentiating grape cultivars, considerable effort has been made in determining the compositions and contents of polyphenolic compounds in grapes, as well in wine (Guerrero et al., 2009a).

Along with the chemical profiling, molecular biology techniques offer powerful analytical tools for grape variety identification and product authentication. In particular, molecular markers are irrespective of environmental factors, free of epistatic interactions and pleiotropic effects, objectively analyzable at all stages of plant growth, and therefore they provide an excellent tool for fingerprinting and assessing genetic variation and relatedness among cultivars of various crops (Kalia, Rai, Kalia, Singh, & Dhawan, 2011). In recent years, several molecular markers has become available for basic and applied studies; random amplification of polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs) and simple sequence repeats (SSRs) are some of the markers developed for germplasm characterisation of different crop plants (Varshney et al., 2007). In particular, SSRs, also known as microsatellites, are useful for many applications in plant genetics and breeding because of their reproducibility, multiallelic nature, codominant inheritance, relative abundance and good genome coverage. They have extensively been used in grape for analysing the genetic diversity of local accessions and exploring pedigree authentication, often in combination with biochemical analyses. Albeit the interesting results reported in literature, no attempts to directly correlate genetic profiles and wine analytical composition have been carried out.

In this work we assessed the phenolic content and composition of wines obtained from native Campania region red grape varieties as Aglianico biotypes (Taurasi, Vulture and Taburno), Piedirosso and Lingua di Femmina. We also carried out the genetic analysis of these cultivars to acquire detailed information for a certification of their production authenticity that combines quality attributes with a clear regional identity.

## 2. Materials and methods

### 2.1. Wines and plant material sampling

Wines were obtained from grapes of *V. vinifera* cultivar 'Piedirosso' (P), 'Cabernet Sauvignon' (CS), 'Merlot' (M), 'Aglianico di Taurasi' (T), 'Aglianico del Taburno' (A), 'Aglianico del Vulture' (V) and 'Lingua di Femmina' (L). All grapes and leaf samples were collected from vineyards located in the area surrounding the city of Benevento (Campania, Italy). These vineyards lay at an altitude ranging between 350 and 650 m above sea level (a.s.l.); the annual mean precipitation received was 850 mm, and the annual mean temperature 13 °C. The soils were both clays and limestone. The vineyards were located in the Taburno DOC area of Campania region (latitude between 41° and 48°N, longitude between 12° and 14°W). Leafs were collected from individual plant. All wines were produced by a conventional winemaking procedure. Briefly grapes were destemmed and crushed, the must was treated with $K_2S_2O_5$ (60 mg/kg of grapes). Fermentation took place at almost 26 °C with indigenous yeast and the cap was immersed twice a day. Maceration of the pomace lasted 18–20 days then the must was pressed and the finished wines obtained. Wines were stored in stainless steel tanks and analysed 6–10 months after the end of the fermentation. Overall, 72 wines were analysed for 18 biochemical parameters.

### 2.2. Biochemical analysis

#### 2.2.1. Standard chemical analyses and spectrophotometric measurements

Tannins were evaluated as described by Ribéreau-Gayon and Stonestreet (1966). Total anthocyanins and Vanillin Reactive Flavans (VRF) were determined according to Di Stefano and Guidoni (1989). Colour intensity and hue were evaluated according to Glories (1984) methods. A Shimadzu UV-1800 (Kyoto, Japan) UV spectrophotometer was used for all data pertaining to the results reported in this article. Photometric accuracy was of ±0.002 Abs and photometric repeatability was less than ±0.001 Abs. All analyses were carried out in triplicate.

#### 2.2.2. HPLC equipment and chemicals

A HPLC Shimadzu LC10 ADVP apparatus was used (Shimadzu Italy, Milan), consisting of a SCL-10AVP system controller, two LC-10ADVP pumps, a SPD-M 10 AVP detector, and an injection system full Rheodyne model 7725 (Rheodyne, Cotati, CA) equipped with a 20 μL loop. All the samples were filtered through 0.45 μm, Durapore membrane filters (Millipore – Ireland) into glass vials and immediately injected into the HPLC system. All chromatographic solvents were HPLC ultra gradient grade and were purchased from Merck (Darmstadt, Germany). Malvidin-3-monoglucoside (Mv-3-glc), *trans*-resveratrol, quercetin (Sigma–Aldrich, Milan, Italy), (+)-catechin hydrate (purity >90%) and (−)-epicatechin (purity ⩾90%) (Fluka, Milan, Italy) standards were used in this study.

#### 2.2.3. Anthocyanins method

For the separation and quantification of anthocyanins a Waters Spherisorb column (250 × 4.6 mm, 4 μm particles diameter) with precolumn (NOVA-PAK C18 20 × 3.9 mm, 4 μm particles diameter) was used. HPLC separation of anthocyanins was carried out according to the OIV Compendium of International Methods of Wine and Must Analysis (2007) with slight modifications. Twenty μl of wine or calibration standards were injected onto the column. The HPLC solvents were: solvent (A) water/formic acid/acetonitrile (87:10:3) v/v; solvent (B) water/formic acid/acetonitrile (40:10:50) v/v. Zero-time conditions were 94% A and 6% B, after 15 min the pumps were adjusted to 70% A and 30% B, at 30 min to 50% A and 50% B, at 35 min to 40% A and 60% B, at 41 min, end of analysis, to 94% A and 6% B. This zero-time solvent mixture was followed by 10-min equilibrium period prior to inject the next sample. The flow rate was 0.80 ml/min. Detection was carried out by monitoring the absorbance signals at 518 nm. Detector sensitivity was 0.01 Absorbance units full scale (AUFS). For calibration the external standard method was used: the calibration curve was plotted for the malvidin-3-monoglucoside on the basis of peak area. The calibration curve was obtained by injecting 5 solutions (in triplicate) containing increasing concentrations of malvidin-3-monoglucoside. The anthocyanins concentrations were expressed as mg/l of malvidin-3-monoglucoside. The identification and assignation of each compounds was confirmed by high-performance liquid chromatography–electrospray ionisation-mass spectrometry (HPLC–ESI-MS) using a Thermo-Finnigan LCQ Advantage spectrometer (Thermo Finnigan, San Jose, CA) equipped with an electrospray ionisation source and an ion trap mass analyser. The ESI-MS detection was performed in positive mode. The parameters of analysis were: capillary temperature 400 °C, capillary voltage −3 V, nebulizer gas flow 1.75 L $min^{-1}$, desolvation gas flow 1 L $min^{-1}$, and spray voltage 5 kV. The analyses were carried out in triplicate.

#### 2.2.4. Trans-resveratrol, catechin, epicatechin and quercetin method

Separation and quantification were carried out by HPLC, as described by Goldberg, Karumankiri, Diamandis, Soleas, and Ng