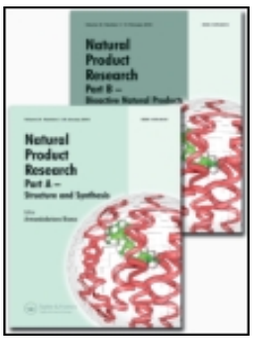

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# Nero d'Avola and Perricone cultivars: determination of polyphenols, flavonoids and anthocyanins in grapes and wines

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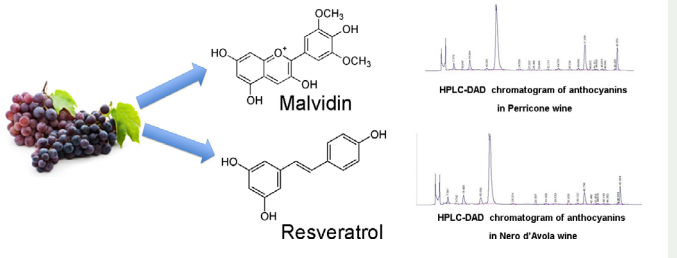
**ABSTRACT**

In 2011 vintage, the evolution of monomer and total anthocyanins, as well as of total flavonoids and polyphenols of grapes and wine of Nero d'Avola and Perricone, varieties cultivated in Sicily, was studied. Anthocyanin profiles are commonly used for grapevine cultivar identification because it is currently accepted that this trait is closely related to their genetic characteristics. The concentration of Nero d'Avola and Perricone anthocyanins was determined by HPLC-DAD.

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**KEYWORDS**
Nero d'Avola; Perricone; polyphenols; flavonoids; anthocyanins profile; HPLC-DAD

## 1. Introduction

Polyphenols are a group of about 8000 compounds identified in various plant species as secondary metabolites and responsible of the defence against ultraviolet radiation or aggression by pathogens (Wink 1997; Pandey & Rizvi 2009). They are often conjugated with one or more sugar residues linked to hydroxyl groups or to an aromatic carbon (Kondratyuk & Pezzuto 2004; Adams 2006).

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In modern life, they result to be very interesting molecules as nutraceuticals and functional foods (Lesschaeve & Noble 2005; Scalbert et al. 2005; Spencer et al. 2008; Alesci et al. 2014; Corsaro et al. 2015; Soares et al. 2015; Cacciola et al. 2016).

These compounds are known for their positive effect on human health, according to epidemiology studies, polyphenols consumption in diet offers protection against heart disease (Beckman 2000) and prevents LDL oxidation, by acting as 'scavengers of free radical', especially peroxide, breaking its formation chain and neutralising them (Vinson et al. 1998; Parr & Bolwell 2000; Kondratyuk & Pezzuto 2004; Arts & Hollman 2005; Graf et al. 2005).

The polyphenol content of various foods is very different, and it is affected by environmental factors as well, the ripeness degree and edaphic factors such as soil type, sun exposure, rainfall (Corona & Pusateri 2004; Manach et al. 2004).

In general, phenols acid content decreases during ripening, whereas amount of total anthocyanins increases (Mattivi et al. 2006). Also, the storage affects the polyphenol content because of the easy oxidation of these polyphenols.

Wines and grapes polyphenolic constituents, classified as flavonoids and non-flavonoids, play a major role in oenology. They are responsible for all the differences between red and white wines, especially for the colour and flavour (Ribéreau-Gayon et al. 2000) and particularly for the taste of bitterness and the tactile sensation of astringency (Lesschaeve & Nobel 2005).

The phenolic compounds of grapes, and mostly the anthocyanins, are widely used for taxonomic purposes, as showed by an extensive bibliography (Scienza et al. 1989; Asselin & Ballester 1992; Castia et al. 1992; Baldi et al. 1993; Calò et al. 1994; Eder et al. 1994; Hmamouchi et al. 1995; Castellarin et al. 2006; Mattivi et al. 2006; Deluc et al. 2007; Dai et al. 2013). All the known varieties show varietal differences among the anthocyanin profiles, and this aspect allows regrouping them in class. For this purpose, the more evident characteristics are the eventual presence of acylated anthocyanins, the ratio anthocyanins acetates – anthocyanins cinnamates, the prevalence of disubstituted or trisubstituted anthocyanins and the relationships among the five non-acylated anthocyanin (Squadrito et al. 2007; Mulinacci et al. 2008).

The main aim of this research was to characterise the total polyphenols, flavonoids and anthocyanins together with the anthocyanins profiles of two Sicilian biotypes of *Vitis vinifera* grapes (Perricone and Nero d'Avola) variety grown in the same place. The determination of these compounds was evaluated in 10 samples of both grapes and wine collected in different periods (Table 1). In a preliminary step, the identification of grape cultivar was determined by DNA microsatellite analysis (unreported data).

As reported by the literature, the different biotypes of Perricone and Nero d'Avola wines are characterised by various anthocyanins profiles (Varvaro et al. 2006; Squadrtito et al. 2007).

**Table 1.** Anthocyanins percentage on total anthocyanins in Perricone grapes.

| | RI | Ago 04 | Ago 11 | Ago 24 | Set 28 |
|---|---|---|---|---|---|
| Delphinidin-3-glucoside | 6.857 | 4.79 | 2.21 | 2.54 | 1.95 |
| Cyanidin-3-glucoside | 8.501 | 2.83 | 1.11 | 1.37 | 2.9 |
| Petunidin-3-glucoside | 9.514 | 11.07 | 8.93 | 5.79 | 7.22 |
| Peonidin-3-glucoside | 12.691 | 8.76 | 8.51 | 8.95 | 17.09 |
| Malvidin-3-glucoside | 15.823 | 49.17 | 63.74 | 54.72 | 58.45 |
| Acetates | 36.826 | 10.56 | 3.79 | 11.56 | 3.18 |
| Cinnamates | 47.290 | 12.8 | 11.69 | 15.06 | 9.2 |

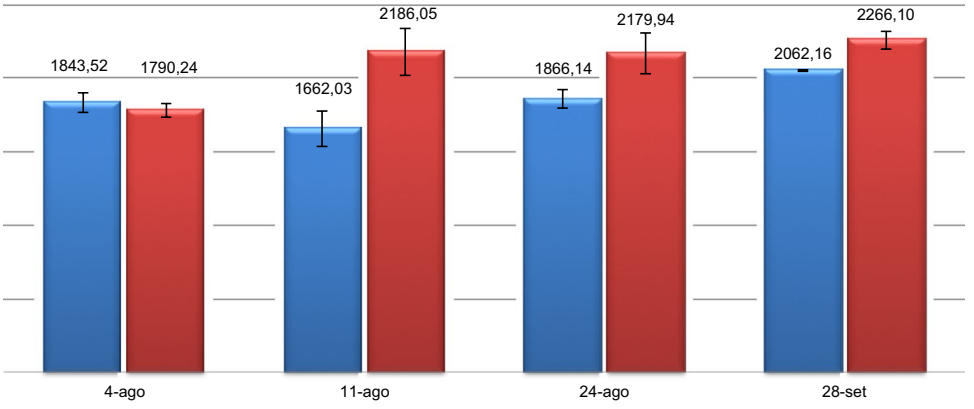


**Figure 1.** Total polyphenols in Perricone (red bars) and Nero d'Avola (blue bars) grapes, expressed in mg/kg of (+) – catechin.

These variability concerning the acetylated anthocyanins percentage, the acetated and cinnamated anthocyanins ratio and the different amounts of the various anthocyanins make the study of the various biotypes very interesting.

## 2. Results and discussion

### 2.1. Total phenolic content

As shown in Figure 1, the concentration of total polyphenols (PT) in the grapes samples increases in relation to the maturity period, with a maximum peak in correspondence of the full maturity (28 September). The maximum PT concentration amounted to 2266.10 ± 59.38 and 2062.16 ± 7.83 mg/kg, respectively, for Perricone and Nero d'Avola grapes. The investigations conducted on wines show that the total polyphenols content maintains a greater concentration in the Perricone wine (2705.20 ± 144.60 mg/L) compared to the Nero d'Avola one (1906.30 ± 49.02 mg/L) (Figure 2). The evaluation of the polyphenols is very interesting; these compounds are responsible for the characteristics, colour and quality of wines. In this study, a difference in both grape and wine highlights that the maceration process occurring during the fermentation has influence only slightly on the extraction of grape polyphenolics into the wine.

### 2.2. Total anthocyanins and flavonoids content

Anthocyanins, which appear during the veraison stage, are accumulated throughout the maturation period and reach their maximum production if the variety is suitable to the environment and mostly if the environmental conditions are consistent.

In Nero d'Avola grapes, the total anthocyanins (TA) content has an upward trend in the later stage of maturation. The higher peak concentration was observed in the sample collected on 28 September (633.60 ± 15.55 mg/kg) which corresponds to the phenol maturity. Instead the higher total anthocyanins concentration in Perricone grape was observed in the sample collected on 24 August (792.09 ± 36.25 mg/kg). The results obtained show that the total anthocyanins content in Perricone grapes is higher than Nero d'Avola ones (Figure 3).

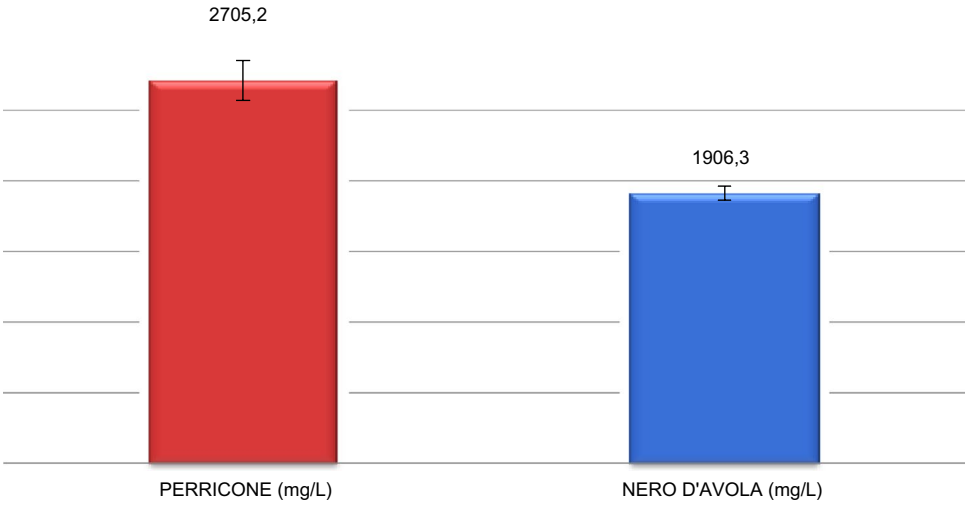


**Figure 2.** Total polyphenols in Perricone (red bars) and Nero d'Avola (blue bars) wines expressed in mg/L di (+) – catechin.

Also the total flavonoids content (FT) is higher in Perricone grapes, and the higher peak concentration (3233.29 ± 347.32 mg/kg) was observed in the last harvest dates. Moreover, Nero d'Avola grapes show lower value in comparison with Perricone grapes, but also for this sample, the maximum peak concentration (2519.22 ± 66.91 mg/kg) was observed in the later stage of maturation (Figure 4).

The analyses carried on the corresponding wines, on the contrary, showed a higher concentration of total anthocyanins in Nero d'Avola sample (369.89 ± 21.515 mg/L) (Figure 5). Probably, this is due to the skin structure of the two cultivars and to the different anthocyanins capacities of extraction from the berries, thanks to the effect of the alcohol produced during fermentation.

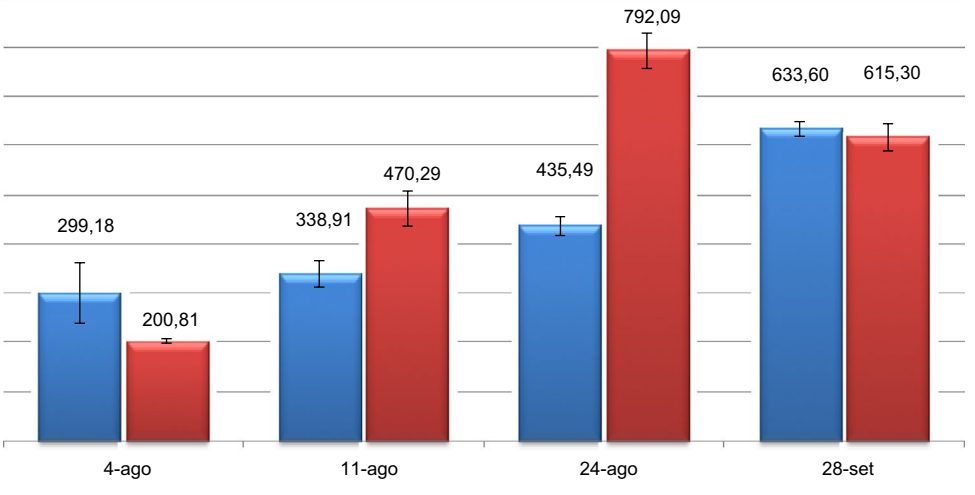


**Figure 3.** Total anthocyanins in Perricone (red bars) and Nero d'Avola (blue bars) grapes expressed in mg/kg of malvidin-3-O-glucoside.

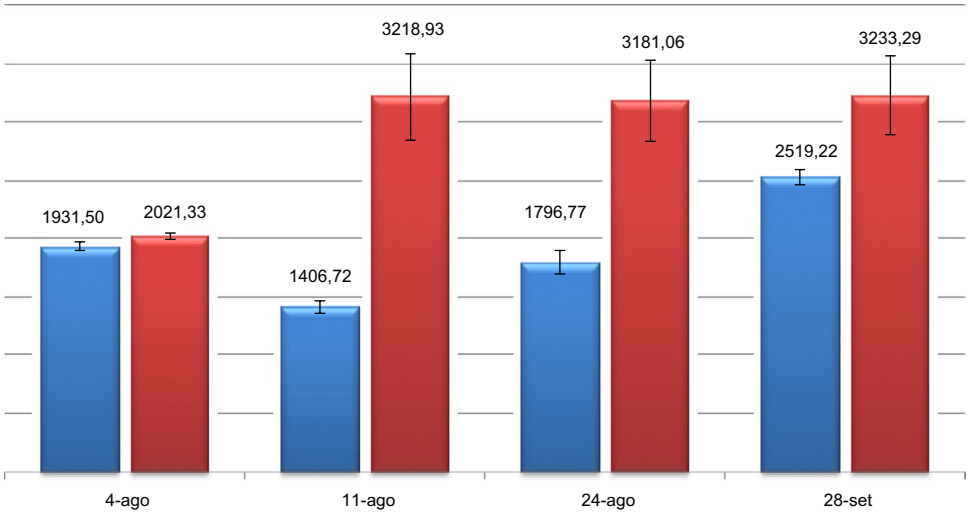


**Figure 4.** Total flavonoids in Perricone (red bars) and Nero d'Avola (blue bars) grapes FT expressed in mg/kg di malvidin-3-O-glucoside.

The high concentration of FT in the Perricone grapes is maintained even after the wine-making, with a value of 3193 ± 145.66 mg/L, while in the Nero d'Avola wine, the FT content is lower (2323.68 ± 93.22 mg/L) (Figure 6).

### *2.3. Anthocyanin profiles analysis by HPLC-DAD*

The results of the grapes and wine anthocyanin profiles and the acetate and the cinnamate values are shown in Tables 2–4 (Figure 1S–4S), respectively.

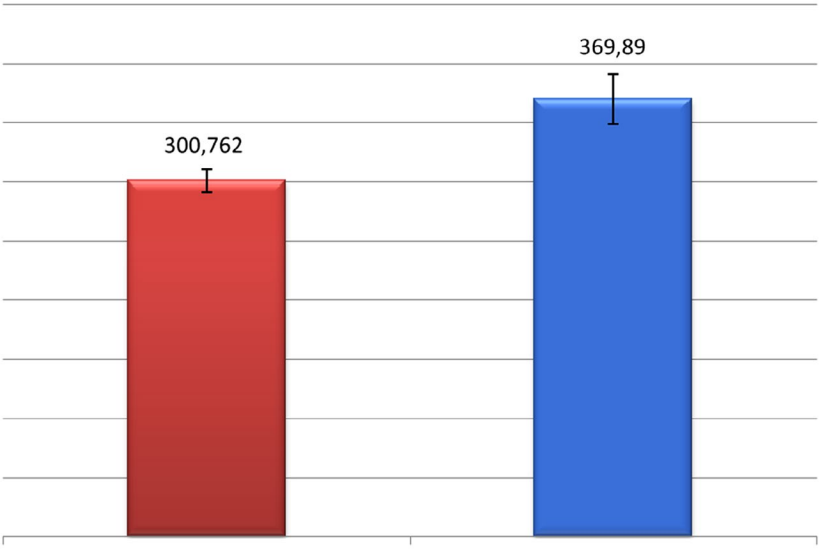


**Figure 5.** Total anthocyanins in Perricone (red bars) and Nero d'Avola (blue bars) wines in mg/L di malvidin-3-O-glucoside.

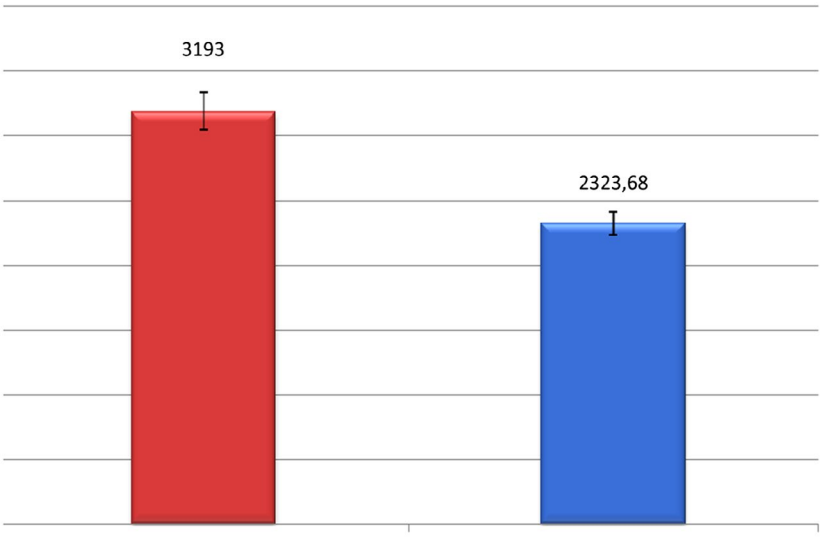


**Figure 6.** Total flavonoids in Perricone (red bars) and Nero d'Avola (blue bars) wines expressed in mg/L di malvidin-3-O-glucoside.

The anthocyanins profile is reported as percentage content of each anthocyanins, and it can help to identify the cultivars.

According to the literature, the main features of Perricone anthocyanin profile is the malvidin prevalence (Squadrtito et al. 2007). The analyses conducted in this study confirm this aspect. In Perricone cultivar, malvidin-3-glucoside (anthocyanin trisubstituted) is the most abundant anthocyanin group, followed by substituted anthocyanins and particularly by petunidin, peonidin, that in grapes could reach percentage of about 20%, delphinidin and cyanidin peonidin.

In Perricone grapes profile, the relationship between the anthocyanins acetates and p-cumarati is always less than 1 (Table 1).

Nero d'Avola, however, has a high percentage of anthocyanins trisubstituted, such as malvidin, petunidin and delphinidin (Table 2).

The wine profiles showed lower anthocyanins values in comparison with the grapes except for the malvidin (Table 3).

## 3. Experimental

See Supplementary materials for: samples and extraction,total phenols determinations, total anthocyanins and flavonoids determinations and anthocyanin profiles of grape and wine by HPLC-DAD.

**Table 2.** Anthocyanins percentage on total anthocyanins in Nero d'Avola grapes.

| | RI | Ago 04 | Ago 11 | Ago 24 | Set 28 |
|---|---|---|---|---|---|
| Delphinidin-3-glucoside | 7.164 | 3.87 | 3.28 | 4.11 | 7.48 |
| Cyanidin-3-glucoside | 9.031 | 1.4 | 0.49 | 0.67 | 2.61 |
| Petunidin-3-glucoside | 10.234 | 9.08 | 7.36 | 7.04 | 7.34 |
| Peonidin-3-glucoside | 13.847 | 6.13 | 3.68 | 3.73 | 8.59 |
| Malvidin-3-glucoside | 15.890 | 50.59 | 52.42 | 47.41 | 43.24 |
| Acetates | 38.887 | 15.44 | 14.4 | 18.94 | 14.65 |
| Cinnamates | 49.051 | 13.48 | 18.36 | 18.08 | 16.07 |

**Table 3.** Anthocyanins percentage in Perricone Nero d'Avola wines.

| | Perricone wine | Nero d'Avola wine |
|---|---|---|
| Delphinidin-3-glucoside | 2.57 | 2.48 |
| Cyanidin-3-glucoside | 0.51 | 0.09 |
| Petunidin-3-glucoside | 4.35 | 5.45 |
| Peonidin-3-glucoside | 3.73 | 1.16 |
| Malvidin-3-glucoside | 52.22 | 59.31 |
| Acetates | 22.04 | 19.32 |
| Cinnamates | 14.57 | 12.19 |

## 4. Conclusion

The determination of the total polyphenols, flavonoids and anthocyanins together with the anthocyanins profiles of two Perricone and Nero d'Avola variety was conducted, and the results are comparable to the ones reported in the literature.

The ripening degree influences the amount of the various phenols fractions of grapes and wine. In both the varieties analysed, the higher total polyphenols concentration is found in the later stages of collection, the phenolic maturity phase, the period that allows the wine-maker to obtain, already in the field, a product with certain wine quality characteristics.

Among the two varieties considered, the Perricone grapes show a higher content of total polyphenols, anthocyanins and total flavonoids than the Nero d'Avola variety. Instead, the Perricone wine maintains a higher content of total polyphenols and flavonoids than Nero d'Avola, but it was found that anthocyanins had a higher concentration in this last wine.

The wines made from both these varieties show high content in anthocyanins, flavonoids and total polyphenols, confirming their antioxidant qualities and health benefits.

The anthocyanins profile takes part to the varietal characterisation of the cultivars. It derives mostly by the genetic profile of the variety and slightly influences by environmental and/or agricultural variables (Mattivi et al. 1990, 2006).

## Disclosure statement

No potential conflict of interest was reported by the authors.

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