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Diversity of Italian red wines: a study by enological parameters, color, and phenolic indices

Simone GIACOSA[1], Giuseppina Paola PARPINELLO[2], Susana RÍO SEGADE[1], Arianna RICCI[2], Maria Alessandra PAISSONI[1], Andrea CURIONI[3], Matteo MARANGON[3], Fulvio MATTIVI[4,5], Panagiotis ARAPITSAS[5], Luigi MOIO[6], Paola PIOMBINO[6], Maurizio UGLIANO[7], Davide SLAGHENAUFI[7], Vincenzo GERBI[1], Luca ROLLE[1]*, Andrea VERSARI[2]

[1] *Department of Agricultural, Forest and Food Sciences, University of Torino, 10095 Grugliasco, Italy*

[2] *Department of Agricultural and Food Sciences, University of Bologna, 47521 Cesena, Italy*

[3] *Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, 35020 Legnaro, Italy*

[4] *Department of Physics, Bioorganic Chemistry Laboratory, University of Trento, 38123 Povo, Italy*

[5] *Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach, 38010 San Michele all'Adige, Italy*

[6] *Department of Agricultural Sciences, Division of Vine and Wine Sciences, University of Napoli Federico II, 83100 Avellino, Italy*

[7] *Department of Biotechnology, University of Verona, 37134 Verona, Italy*

* Corresponding author. E-mail: luca.rolle@unito.it

ABSTRACT

An extensive survey was conducted on 110 Italian monovarietal red wines from a single vintage to determine their standard compositional, color, and phenolic characteristics, analyzing more than 35 parameters evaluated through methods commonly used in the wine industry. 'Primitivo' achieved the highest average alcohol strength (15.4 % v/v) and dry extract values, while 'Cannonau' showed the lowest total acidity. 'Corvina' had the lowest phenolic content (1065 mg/L by Folin-Ciocalteu assay), remarkably different from the highest found in 'Sagrantino' (3578 mg/L), the latter being also the richest variety in both proanthocyanidins and vanillin-reactive flavanols. 'Teroldego' wines were the richest in both total and monomeric anthocyanins (702 and 315 mg/L, respectively), followed by 'Aglianico' and 'Raboso Piave', while 'Corvina', 'Nebbiolo', and 'Nerello Mascalese' were the poorest. 'Montepulciano' and 'Sangiovese' showed intermediate values for the majority of the parameters analyzed. A multivariate PCA-DA approach allowed achieving both a classification of the different wines as well as the discrimination of 'Sangiovese' wines produced in two regions (Emilia Romagna and Toscana) that returned a 42–66 % success rate depending on the zone considered. Taking into account the number and diversity of the wines analyzed, a correlation study helped in better understanding the underlying relations between the most common and widespread analytical techniques for phenolic and color determinations.

Keywords: autochthonous grape varieties; phenolic compounds; tannins; antioxidant capacity; red wine; UV-Visible spectrophotometry; multivariate analysis; D-Wines collaboration.

Highlights

- 110 monovarietal red wines, produced from 11 Italian varieties, were analyzed
- A correlation study on basic, phenolic, and color parameters was performed
- The high variability among wine groups evidenced distinctive varietal traits
- Strong correlations between phenolic and flavanol indices were found
- 'Nebbiolo', 'Teroldego' and 'Sagrantino' were well discriminable from other wines

1. INTRODUCTION

The wine sector is a main economic agricultural activity worldwide and, among the factors affecting the wine style, the role of grape (*Vitis vinifera* L.) varieties is of primary importance. It is well known that many grape varieties can be used for the production of wine, affecting its composition and sensorial quality. This aspect is particularly important in Italy, a country that owns one of the richest ampelographic heritages worldwide, and that presents more than 600 cultivated grape varieties registered (Bavaresco, Pecile, & Zavaglia, 2014).

Red wine is a source of phenolic compounds, including flavanols – such as catechins and oligomeric and polymeric proanthocyanidins (PAs, also known as condensed tannins) with variable degree of galloylation – that are extracted from the grapes during winemaking, leading to differences in the wine composition (Mattivi, Vrhovsek, Masuero, & Trainotti, 2009; Garrido & Borges, 2013; Unterkofler, Muhlack, & Jeffery, 2020). Other main compounds, such as anthocyanins and flavonols, have also evidenced a great quali-quantitative variability among grape varieties and, also depending by their extraction and stabilization, are pivotal for wine quality (Mattivi, Guzzon, Vrhovsek, Stefanini, & Velasco, 2006; Unterkofler et al., 2020). Grape phenolics have gained attention because of their *in vitro* and *in vivo* activities (Guilford & Pezzuto, 2011) and they are thought to be associated with the health benefits deriving from a moderate wine consumption (Cooper, Chopra, & Thurnham, 2004). Moreover, these compounds play a key role in determining red wine longevity, color, and mouthfeel (de Freitas & Mateus, 2012; Ma, Guo, Zhang, Wang, Liu, & Li, 2014; Paissoni, Waffo-Teguo, Ma, Jourdes, Rolle, & Teissedre, 2018), as well as to determine their authenticity (Versari, Laurie, Ricci, Laghi, & Parpinello, 2014; Palade & Popa, 2018). Although the wine phenolic content is affected by both grape variety and vintage, together with other viticultural, soil, and climatic factors, the phenolic profile is mostly dependent on the grape variety (Gómez Gallego, Sánchez-Palomo, Hermosín-Gutiérrez, & González Viñas, 2013; Heras-Roger, Díaz-Romero, & Darias-Martín, 2016; Sartor, Caliari, Malinovski, Toaldo, & Bordignon-Luiz, 2017), and for this reason it can be helpful to discriminate the corresponding wines. Nowadays there is an increasing interest in assessing the varietal origin of wines, due to its economic importance and the occurrence of several cases of fraud and mislabeling (Villano et al., 2017).

Spectrophotometric methods for wine phenolic content and color assessments are often used for routine analysis by winemakers and are valuable to researchers as well, based on its ease of use as a routine, rapid, and cost-effective analytical technique (Luque de Castro, González-Rodriguez, & Perez-Juan, 2005; Aleixandre-Tudo, Buica, Nieuwoudt, Aleixandre, & du Toit, 2017). Often, the traditional spectrophotometric assays provide a good deal of valuable information on the amount and nature of wine phenolic compounds, comparable to HPLC (Vrhovsek, Mattivi, & Waterhouse, 2001). These methods were used also with the aim to correlate the chemical and sensory properties of wines, as it was found for several parameters linked to wine visual appearance (Parpinello, Versari, Chinnici, & Galassi, 2009). In the context of chemical-sensory correlations, it is also of great interest to find rapid tools to evaluate wine astringency and its relationship with tannins. According to Kennedy, Ferrier, Harbertson, & Peyrot des Gachons (2006), spectrophotometric tannins assays, such as the Harbertson, Picciotto, & Adams (2003) tannin assay, showed the highest correlations with wine astringency, followed by phloroglucinolysis. Similarly, the relationship between tannin quantification and perceived wine astringency was assessed for the methyl cellulose precipitable tannin (Mercurio & Smith, 2008) and Adams-Harbertson tannin assays, and the latter method showing the best correlation with astringency (Mercurio & Smith, 2008). Total phenolic and tannin concentrations can also drive the decisions for marketing strategies of wine grade allocations depending on wine style and market place. For example, analysis of data collected from Shiraz and Cabernet Sauvignon red wines produced in Australia (1643 wines from 2005, 2006, and 2007 vintages) showed a positive trend toward higher wine grade allocation for wines with higher concentrations of both total phenolics and tannins (Mercurio, Dambergs, Cozzolino, Herderich, & Smith, 2010). In another study, Fanzone, Peña-Neira, Gil, Jofré, Assof, & Zamora (2012) focusing on Argentinean red wines (Malbec and

Cabernet Sauvignon) confirmed that the wines of greater commercial value, having the best visual and gustatory scores, matched with higher levels of phenolic parameters determined by spectrophotometric methods.

To date, the physical-chemical characteristics of most monovarietal red wines produced in Italy have gained limited attention by researchers. Few systematic studies have been published (Mattivi & Nicolini, 1997; Mattivi, Zulian, Nicolini, & Valenti, 2002), and this lack of information needs to be fulfilled. With this purpose, a large national project has been implemented to investigate the diversity of tannins in Italian red wines, and several results arising from this effort have recently been published, focusing on metabolomics, MIR spectroscopy characterization, and sensory assessment of a comprehensive set of wines (Parpinello et al., 2019; Arapitsas et al., 2020; Piombino et al., 2020; Pittari et al., 2020).

This study reports data on the physicochemical characteristics of monovarietal Italian red wines with the aim to elucidate their diversity in relation to the grape variety from which they derive. The choice of a wide range of rapid analyses proposed in scientific literature and widespread in the winemaking industry, determining the basic, phenolic, and color traits of the 110 red wine samples tested, allowed to highlight reference values for quality indices highly demanded by winemakers, the investigation of relationships among the different parameters studied, and the multivariate classification of these wines using a PCA-DA approach. Furthermore, a critical insight on the selected analytical determinations provided improved understanding of the obtained findings.

2. MATERIALS AND METHODS

2.1. Samples

A total of 110 (*N*) monovarietal red wines, vintage 2016, were collected directly from commercial wineries across different regions of Italy (**Figure S1**). The wines produced from the following eleven grape varieties were considered: 'Sangiovese' (*n*=19, of which 7 samples from Toscana and 12 from Romagna regions), 'Nebbiolo' (*n*=11), 'Primitivo' (*n*=11), 'Teroldego' (*n*=11), 'Aglianico' (*n*=10), 'Raboso Piave' (*n*=10), 'Sagrantino' (*n*=10), 'Cannonau' (*n*=9), 'Montepulciano' (*n*=9), 'Corvina' (*n*=7), and 'Nerello Mascalese' (*n*=3). These varieties were selected according to their importance for each Italian region and their phenolic characteristics. All these varieties accounted together for about 44 % of red grapevine cultivated area in Italy (Arapitsas et al., 2020; OIV, 2018). The wines were sampled in early year 2017, adjusted to 50 mg/L of free sulfur dioxide, and stored in glass bottles sealed with Select Green 500 corks (Nomacorc, Rivesaltes, France) until analysis. All wines were produced from a single grape variety, and were not blended with wines from other regions and therefore, although not representing commercially available products, the wines are the actual expression of their own varietal uniqueness.

2.2. Analytical methods

For all analyses, suitable analytical-grade reagents were provided by Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Deionized water was produced using a DEIONEX TWO equipment (Appen.Lab., Torino, Italy).

All wines were analyzed for the following parameters according to existing analytical methods: polymeric fraction of color and copigmentation (Boulton, Neri, Levengood, & Vaadia, 1999); iron reactive tannins (tannins-Fe), iron reactive phenolics (phenolics-Fe), small polymeric pigments (SPP), and large polymeric pigments (LPP) (Harbertson, Picciotto, & Adams, 2003); buffer capacity

(Dartiguenave, Jeandet, & Maujean, 2000); total aldehydes (Crowell & Guymon, 1963); antioxidant activity by ABTS$^{\bullet+}$ (2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid radical cation) assay (Re, Pellegrini, Proteggente, Pannala, Yang, & Rice-Evans, 1999); methyl cellulose precipitable (MCP) tannin assay (Mercurio & Smith, 2008), total anthocyanins, total flavonoids, and monomeric anthocyanins (Di Stefano, Cravero, & Gentilini, 1989); total phenols (Folin-Ciocalteu reagent) (Singleton & Rossi, 1965); proanthocyanidins and vanillin-reactive flavanols (PROC and VAN, respectively; Porter, Hrtisch, & Chan, 1986; Sun, Ricardo-Da-Silva, & Spranger, 1998; Di Stefano et al., 1989). Furthermore, the UV-Vis spectra ranging 190-700 nm was acquired on wines diluted 500 times with water, and the absorbances at 230, 280, and 340 nm were extracted. The undiluted wine was also acquired in the Vis region (370-700 nm) to calculate the color intensity, hue (also indicated as shade), and CIELab parameters (L*, clarity; a*, red-green color component: b*, blue-yellow color component; C*, chroma, H*, hue angle) by adapting OIV-MA-AS2-11 and OIV-MA-AS2-07B methods (OIV, 2016), and to extract the absorbance values at 420, 440, 520, 580, and 620 nm. These spectrophotometric readings were carried out using a Cary 60 spectrophotometer (Agilent Technologies, Santa Clara, USA) on 10 mm optical path length cuvettes, except for Vis color measurements that were conducted on undiluted wine using 1 mm path length cuvettes. In this last case, the color intensity value was normalized on a path length of 10 mm.

The basic compositional wine parameters were also analyzed. Alcohol, residual reducing sugars, glycerol, titratable acidity, volatile acidity, pH, and total dry extract were determined using a commercial WineScan™ analyser (FOSS A/S, Hillerød, Denmark) composed by an FTIR interferometer (Fourier Transform Infrared Spectroscopy technique) and the integration software (Foss Integrator) provided with built-in calibration curves. This technique is now widespread as a rapid tool for routine wine analysis, and its technical specifications and performances are described by OIV (2010). Before analysis, the samples were centrifuged (2700 $g \times 5$ min) to remove turbidity and CO_2, if present. From the obtained results, net dry extract was calculated by subtracting the residual reducing sugars content from the total dry extract value.

2.3. Data processing, visualization, and multivariate analyses

Clustered heat map (using Ward hierarchical clustering method) on scaled data, boxplot, and scatter plot visualizations were produced using R software (version 3.6.2; R Foundation for Statistical Computing, Vienna, Austria) plus packages ‘pheatmap’, ‘ggplot2’, and ‘ggpubr’. ANOVA statistical analysis was used to underline significant differences at p-levels of 0.05, 0.01, and 0.001. Tukey HSD post-hoc test ($p<0.05$) was also applied to evaluate significant differences among wine groups. Furthermore, Pearson correlation coefficients were calculated among parameters.

Principal Component Analysis (PCA) and Discriminant Analysis (DA) techniques were used as tools to attempt the classification of wines based on the relationship between grape variety and wine composition. In order to overcome the constraint of requiring more objects (i.e. samples) than features (i.e. variables), the PCA-DA approach was used, which reduces the data dimensionality using PCA components prior to running DA. To this aim, the physicochemical data were mean centered and scaled to the same variance by standardization throughout all multivariate statistical procedures. In particular, PCA is an unsupervised technique that reduces the dimensionality of the response matrix to few new principal components (PCs) and was used, after varimax rotation, to examine the hidden structure of the dataset, to evidence correlations between observations and variables in the data, and to highlight possible groupings of samples and outliers (Esbensen, Guyot, Westad, & Houmoller, 2002; Naes, Isaksson, Fearn, & Davies, 2002). DA was then used to attain a classification model whereby the wine samples were categorized according to variety and the classification performance was evaluated by comparing the number of correctly assigned objects to their total number. The predictive models were validated with full-cross-validation using XLStat software (version 2018.3; AddinSoft, Paris, France).

3. RESULTS

The 110 monovarietal Italian red wine samples were analyzed in terms of basic and phenolic composition as well as of color characteristics. **Figure 1** represents the heat map showing clustering and correlations among the whole set of parameters tested across the 110 wines. Six main clusters were found as follows: (i) alcohol, dry extract and its components; (ii) acidity traits with the exception of volatile acidity; (iii) main total phenolics and flavanols indices together with antioxidant activity; (iv) color parameters, anthocyanins, visible spectral data (in absorbance units), and anthocyanin combination indexes (copigmentation and polymerization); (v) CIELab coordinates (plus small polymeric pigments, SPP); (vi) total aldehydes. This latter parameter was close to the group represented by CIELab parameters plus SPP. While the aldehyde content showed a high variability (range 7−194 mg/L across all data), the Pearson correlation coefficients (r) with any of the other analyzed parameters were below 0.50 (on absolute values), therefore explaining the clustering behavior.

3.1. Wine basic composition

The first assessment carried out on wines was the analysis of the standard chemical parameters. The samples variability in terms of alcohol content, pH, titratable acidity, and net dry extract is shown in **Figure 2**, while the underlying data is included in **Table S1**. The wines showed an average alcohol content of 13.9 % v/v (**Figure 2a**), ranging across 11.4 % and 16.6 % v/v. 'Corvina' wines evidenced the lowest values (average 12.1 % v/v), while 'Primitivo' wines had the highest (15.4 % v/v). Considering the average values, these two varieties enclose all the others for a high number of compositional parameters, including dry extract and glycerol.

Regarding the total dry extract (average 31.5 g/L; **Figure 2b**), variable behaviors were evidenced in the samples, with 'Sangiovese' (Toscana), 'Teroldego', and 'Nebbiolo' showing the lowest intragroup variability. Residual reducing sugars and glycerol represent a good share of the total dry extract, and, particularly, a significant correlation in the analyzed wines was found (0.54 and 0.69, $p<0.01$, respectively; correlation data available in **Table S2**). Residual reducing sugars content was generally not higher than 2.5 g/L (**Table S1**) with the exception of 'Primitivo' (5.4 g/L), 'Aglianico' (2.8 g/L), and 'Raboso Piave' (3.1 g/L) that showed mean values above the population average. In particular, 'Primitivo' wines presented contents above this threshold (2.5 g/L), and two samples reached 7.6 g/L and 20.1 g/L. This latter clearly underwent an incomplete alcoholic fermentation as testified by an alcohol degree (15.8 % v/v) and volatile acidity (0.45 g/L). Glycerol was highly correlated with alcohol (r=0.68, $p<0.01$), and also in this case 'Corvina' and 'Primitivo' wines clearly differed from the majority of the others.

Concerning titratable acidity, two distinct groups ($p<0.05$) (**Figure 2c**; **Table S1**) emerged from the statistical analysis: the highest values were found in 'Raboso Piave' (average 7.7 g/L as tartaric acid), 'Aglianico' (7.2 g/L), and 'Primitivo' (7.0 g/L) wines, while all others were grouped with average values ranging between 4.9 ('Cannonau') and 5.5 g/L ('Nebbiolo'). pH values partly reflected these trends (**Figure 2d**), leading to a significantly negative correlation with titratable acidity (r= –0.46; $p<0.01$). However, the parameter most strongly correlated with titratable acidity was the buffer capacity (r=0.76; $p<0.01$).

3.2. Phenolic compounds

The monovarietal wines tested owned distinctive contents of phenolic compounds (**Figure 3**, numerical data available in **Table S3**). The total phenols index (**Figure 3a**), evaluated through the Folin-Ciocalteu assay and expressed as (+)-catechin, was able to subdivide roughly the dataset in four main groups. 'Corvina' wines marked by far the lowest amount of total phenolic compounds (1065 ± 417 mg/L); followed by 'Sangiovese' (Toscana), 'Cannonau', 'Montepulciano', and 'Nerello

Mascalese' (average values ranging between 1945 and 2033 mg/L); 'Sangiovese' (Romagna), 'Teroldego', 'Primitivo', and 'Raboso Piave' showed intermediate results (average values ranging 2202–2497 mg/L), while 'Nebbiolo', 'Aglianico', and 'Sagrantino' were found at the higher end of the dataset (average values ranging 2841–3578 mg/L). It is noteworthy that all the wines produced with 'Sangiovese' grapes averaged 2107 ± 545 mg/L, but a somewhat large, though non-significant (p>0.05), difference between the two growing zones considered (Toscana and Romagna) was found for this parameter.

Overall, the behavior reported for the results obtained by Folin-Ciocalteu assay characterizes that observed for the other phenolic indices, as all these parameters are positively and highly correlated among them (**Table S4**), excepting anthocyanins.

3.2.1. Flavanol composition

Several spectrophotometric indices were used for the evaluation of the flavanol content and composition of the monovarietal wines. Among them, proanthocyanidins (PROC) and vanillin (VAN) assays were chosen for their specificity to high-molecular weight tannins and low-molecular weight flavanols (plus terminal units of tannins), respectively, therefore contributing to obtain comprementary information on the flavanol characteristics (Vrhovsek et al., 2001). The results shown in **Figure 3c-d** (and the average data in **Table S3**) tend to confirm the general trends already found for the total phenols index. For the vanillin assay, which estimates the monomers and terminal units, average values were in the range from 528 to 2942 mg (+)-catechin/L, while for the proanthocyanidins assay, which measures tannins, they ranged between 1309 and 5371 mg cyanidin chloride/L (**Figure 4a**).

Despite the two assays are different, the results obtained were found to be highly correlated (Pearson coefficient r=0.89; p<0.01; **Table S4**) and, as above mentioned, led to group the wines in a way similar to that of total phenols. These considerations are valid also for the total flavonoids index, and for Fe-reactive tannins and phenolics. Concretely, both proanthocyanidins and vanillin assays correlate well with these other phenolic and flavanol indices (r=0.78-0.95; $p<0.01$). Concerning the two iron-mediated assays, the monovarietal wines tested were in the range 118−1750 mg/L for tannins-Fe, and 888−3737 mg/L for phenolics-Fe (data not shown).

Total tannins were also evaluated by means of methyl cellulose precipitable tannins (MCP) assay and expressed as mg (+)-catechin/L (**Figure 3b**). 'Corvina' samples were characterized by the lowest tannin content (533 ± 319 mg/L), while 'Sagrantino' displayed by far the higher tannin amount (2965 ± 498 mg/L). All the other wines tested were grouped together ($p>0.05$) and showed average values between 1341 and 2043 mg/L.

3.2.2. Anthocyanin content and polymerization degree

According to **Figure 1**, the parameters related to anthocyanin data were clustered together with spectophotometric data acquired in the region 340−620 nm and also with the color fraction related to polymeric forms and copigmentation. Limited Pearson correlations (r<0.50; **Table S4**) were found among total anthocyanins and the phenolic indices mentioned in the previous sections. Among them, a positive correlation of total anthocyanins index was found with the total flavonoids index. Although these two indexes were determined on the basis of the same spectra obtained by the acquisition of wines diluted with an ethanol:water:hydrochloric acid solution, the Pearson correlation coefficient is feeble (r=0.35; $p<0.01$) with respect to the relations previously found among total phenolics and some flavanols parameters. Furthermore, another poor positive correlation (r=0.24; $p<0.05$) was found between total anthocyanins and total phenols (Folin-Ciocalteu assay) indexes. The lack of strong correlations with the phenolic indexes underlines the high impact of tannin composition on the phenolic content in wines, and the need to perform separate analysis for the different classes of flavonoids.

When looking at the total anthocyanin content of the wines (**Figure 3e**), the range of the values was quite broad, with ‘Teroldego’ showing values 7 times higher than ‘Corvina’ wines that were found at the low end of the range alongside ‘Nebbiolo’ and ‘Nerello Mascalese’.

On average, the monomeric anthocyanin content of the wines accounted for the 48 % of the total anthocyanins (**Table S3**). Although a significant strong correlation between monomeric and total anthocyanin indexes was found ($r=0.90$; $p<0.01$), the distribution of monomeric anthocyanin contents seemed slightly variable when compared to the one found for total anthocyanin content (**Figure 4b**), in particular for ‘Sagrantino’, ‘Primitivo’, ‘Montepulciano’, and the group of ‘Sangiovese’ and ‘Cannonau’, which showed a distinct positioning on the y-axis, although these differences were not significant ($p>0.05$; **Table S3**).

Notably, the monomeric anthocyanin percentage (with respect to total contents; **Table S3**) evidenced stronger correlations than both total or monomeric anthocyanin contents when related to flavanols richness: total flavonoids ($r=-0.39$; $p<0.01$) and proanthocyanidins ($r=-0.33$; $p<0.01$) were the phenolic-flavanol parameters mostly correlated with the monomeric anthocyanin percentage. However, probably due to the low correlation factor, variable situations were shown depending by the variety: ‘Sagrantino’ wines presented both the highest phenolic contents and the lowest monomeric anthocyanin percentage (**Figure 3a-f**), and a similar situation was also found in ‘Nebbiolo’, whereas ‘Aglianico’, ‘Raboso Piave’, and ‘Sangiovese’ Toscana showed an opposite trend. Therefore, these results indicate that a definite trend for these parameters was not evidenced among the tested varieties.

3.3. Wine color parameters

The values of CIELab, color intensity, and hue parameters in the different wines studied are reported in **Table S5**, while a graphical representation of L* *vs* a* components and a visual estimation of the average color distribution for the wines considered is available in **Figure S2**. Wines showed average color intensities in the range from 4.02 AU ('Corvina') to 14.82 AU ('Teroldego'), in agreement with the total anthocyanins index. The 'Corvina' wine was also at the higher limit of the distribution for color hue (average value of 1.05), but in this case the lowest average value corresponded to 'Raboso Piave' wine (0.59).

Although the main wine color parameters are derived by a calculation from the same spectra data in the visible region, according to **Figure 1** the CIELab parameters are grouped separately from the color intensity and other absorbance values. In our dataset, strong positive correlations (r=0.65 to 0.97; $p<0.01$; **Table S6**) were found among L* (lightness), a* (red-green color coordinate), b* (yellow-blue color coordinate), C* (chroma), and H* (hue angle) color components, which in turn all of them are negatively correlated (r= –0.78 to –0.91; $p<0.01$) with the color intensity. In fact, 'Teroldego' wines showed the lowest values of the CIELab coordinates (**Table S5**). Color hue was not included in **Figure 1** because it is an absorbance ratio (420/520 nm), however, this parameter was correlated at $p<0.01$ with the previously cited color components, excepting for a* (red-green color component). Regarding the relationships with other color indices, it is well known that CIELab coordinates are obtained by applying a non-linear transformation of the (linear) *x, y, z* tristimulus values that describe color perception at the retinal level, and thus the CIELab system suffers of non-linearity in the blue and red regions of the spectrum (Roy Choudhury, 2015).

The total anthocyanin content is correlated with the color intensity (r=0.72; $p<0.01$; **Table S6**) and is negatively correlated with CIELab components (r= –0.61 to –0.69; $p<0.01$) and color hue (r= –0.43; $p<0.01$), with stronger correlation coefficients compared to those obtained for the monomeric anthocyanins index.

The color fractions related to polymeric forms and copigmentation are two interesting aspects of wine color, especially considering the age of the wines sampled (about 6 months after the beginning of fermentation). A positive correlation of these values with the color intensity (r=0.89 and 0.54, respectively; p<0.01; **Table S6**) and with the absorbance at 520 nm (r=0.85 and 0.53, respectively; p<0.01) was found. However, an important variability of the polymeric color and copigmentation was observed in the analyzed wines when expressed as percentage (data based on the absorbance values at 520 nm; **Table S5**). For instance, 'Raboso Piave' accounted for the lowest polymeric color share (32 %; average of individual observations), a fact that could explain the very low color hue previously found. 'Corvina' wines had the highest polymeric color share (66 %) and the lowest copigmentation share (17 %), as opposed by the highest values found for 'Nerello Mascalese' (54 %).

3.4. Classification of the wines using a PCA-DA approach

3.4.1. Principal Component Analysis (PCA)

The dataset consisted of 110 red wines and 36 physicochemical parameters, including the following three ratios: 230/280 nm, Tannins-Fe/Anthocyanin, and Total/Monomeric anthocyanins. Although the X-matrix was reduced to four principal components (PC_{1-4}) that globally explained the 74 % of total variability, there was little visual grouping according to variety-geographical origin based on the first two PCs (PC_{1-2}= 53 % of variance explained) (data not shown). This result can be due to the high number of grape varieties (n=11), lack of equality in group sizes (e.g. 'Nerello Mascalese' with only 3 samples), and the overall limited sample size. The PC_1 (**Figure 5**) was mainly characterized by the direct spectrophotometric readings related to phenolic compounds (factor loading >0.6: 340 nm, 420 nm, 440 nm, 520 nm, 580 nm, 620 nm, polymeric color, and copigmentation), whereas the PC_2 accounted for selected chemical assays related to polyphenols as well (factor loading >0.7: tannins-Fe, phenolics-Fe, ABTS, total flavonoids, total phenols (Folin-Ciocalteu), proanthocyanidins assay, vanillin assay). The contribution of 280 nm was relevant on both PC_1 (0.64) and PC_2 (0.73). Furthermore, as expected, high correlation between several parameters occurred (**Figure 5; Table S4**) and this multicollinearity (evidenced in **Figure 5**) allowed a reduction of variables suitable for DA.

3.4.2. Discriminant analysis (DA)

DA (**Figure 6**) was used as first attempt to classify the red wines according to their grape variety, except for 'Nerello Mascalese' wine that was removed from the dataset due to the limited number of samples available (n=3). In order to reduce the data dimensionality, the PCA-DA approach was used, therefore using $PCA_{1\text{-}4}$ scores as matrix to run LDA. **Table 1** shows the results of the LDA classification, presented as confusion matrix that displays the probability of membership for each class, as well as the predicted class for each sample, with each row showing the instances in a predicted class, and each column representing the instances in an actual class. The overall correct classification for full-cross-validation was encouraging (ca. 61.7 %) with the better assignment for 'Nebbiolo' (100 %), 'Teroldego' (91 %), 'Sagrantino' (90 %), and 'Corvina' (86 %). The 'Sangiovese' wines (n=19) were divided in two sub-groups ('Sangiovese' Romagna n=12; 'Sangiovese' Toscana n=7) considering the indeterminacy of its origin, disputed between Romagna and Toscana. The 'Sangiovese' from Toscana showed the same probability (about 45 %) to be correctly classified or instead confused with 'Sangiovese' from Romagna. Similarly, also 'Cannonau' wine was confused up to 55 % with 'Sangiovese' from Romagna.

The LDA plot of Italian monovarietal red wines, representing wine scores by centroids as well as variables, clearly showed five separated groups (**Figure 6**): a main cluster of six wines ('Sangiovese' Toscana, 'Sangiovese' Romagna, 'Aglianico', 'Primitivo', 'Raboso Piave', and 'Cannonau'), another overlapping between 'Montepulciano' and 'Corvina', whereas 'Nebbiolo', 'Teroldego', and 'Sagrantino' wines were most effectively discriminated.

4. DISCUSSION

4.1. Wine variability in terms of phenolic estimation by spectrophotometric indices

In red wine production, the quantification and characterization of phenolic compounds, particularly tannins, is of great interest for winemakers, and although further research into the structure and function of tannins with advanced analytical techniques is important, there is also a need for rapid, simple, and robust assays to evaluate the phenolic content in wine. To date, several spectrophotometric methods for the quantification of phenolic compounds have been developed, and those herein selected are well known and widely used in both research and quality control laboratories. Although these methods are inter-correlated, each of them presents advantages and limitations that are discussed below to improve the understanding of the findings presented in this study.

The absorbance at 280 nm is characteristic of the benzene cycles of most phenolics, except cinnamic acids and chalcones (Lorrain, Ky, Pechamat, & Teissedre, 2013). Conversely, some non-phenolic compounds −such as amino acids, proteins, ammonium sulfate, and methyl cellulose (Mercurio & Smith, 2008)− can also absorb at 280 nm causing overestimation, which can be corrected for 4 index units (Somers & Evans, 1974). Similarly, the Folin-Ciocalteu assay (FC) −based on oxidation-reduction reactions in which polyphenols are oxidized− is affected by interferences of several compounds, including sodium bisulphite, reducing sugars, ascorbic acid, some transition metals, and reducing amino acids (tryptophan and tyrosine) (Granato, Sousa Santos, Galvão Maciel, & Sávio Nunes, 2016). As shown in this study, the direct, fast measurement of absorbance at 280 nm (after wine dilution) was the parameter best correlated with the Folin-Ciocalteu assay ($r=0.91$; $p<0.01$; **Table S4**), indicating that the two indices can be used to estimate the total content of phenolic compounds: although these do not have the same numerical value, they respond similarly to variations in phenolic content.

4.1.1. Spectrophotometric assessment of flavanol composition and its linkage with anthocyanin evolution

The widely used proanthocyanidins assay (PROC), which exploits the Bate-Smith reaction, is based on proanthocyanidin depolymerization through the breakdown of their intra-flavan bonds in an acidic medium at high temperature. This assay specifically measures proanthocyanidins and not monomeric catechins (Vrhovsek et al., 2001; Granato et al., 2016) neither hydrolysable tannins (Herderich & Smith, 2005). Instead, the Vanillin assay (VAN) is sensitive to both free flavan-3-ols and terminal units of the proanthocyanidin polymers (Butler, Price, & Brotherthon, 1982). Moreover, the VAN values have been reported to decrease with increasing the polymerization degree of flavanols in red wines (Schneider, 1995; Gambuti, Rinaldi, Ugliano, & Moio, 2012; Picariello, Gambuti, Picariello, & Moio, 2017). For this reason, Gambuti, Picariello, Rinaldi, & Moio (2018) suggested that the 'VAN/monomeric anthocyanins' and 'tannins/anthocyanins' ratios are useful to evaluate (and drive?) the wine evolution under low oxygen exposure during storage. From the data shown in **Table S3**, we can report that 'Sagrantino' wines have an average VAN/monomeric anthocyanins ratio of about 44 (average of individual observations), the second highest value after 'Nebbiolo' (VAN/monomeric anthocyanins of 55), the latter being a variety with a content of flavanols similar to that of the former but poorer in anthocyanins. The most and least-performing varieties for the anthocyanin content, 'Teroldego' and 'Corvina' wines, have an average VAN/monomeric anthocyanin ratio of 3 and 10, respectively, showing a remarkable difference from 'Sagrantino' and 'Nebbiolo', a fact mainly due to the richness in VAN-reactive flavanols.

In wine phenolic analysis, the ratio between the results of vanillin and proanthocyanidins assays (VAN/PROC) is considered a qualitative polymerization index (Vrhovsek et al., 2001) that could give a quick insight also on the reactivity of these flavanol forms. The calculation of the VAN/PROC ratio (from **Table S3** data) shows an overall average value of 0.41 (*N*=110). 'Sagrantino' presented the highest average value (0.55; average of individual observations) shortly followed by 'Aglianico' (0.52). The other wines were mainly positioned in the VAN/PROC range of 0.39–0.44, with the exception of 'Teroldego', 'Primitivo', 'Montepulciano' (all 0.35), and 'Raboso Piave' (0.36). This aspect is important for wine development, and needs to be carefully monitored and adjusted through enological operations.

4.1.2. Relations among phenolic characteristics and antioxidant activity

The ABTS assay, which measures the overall reducing capacity of wine, showed satisfactory ($p<0.01$) and positive correlations with all the phenolic parameters tested (except monomeric anthocyanins), peaking with both the absorbance at 280 nm and the Folin-Ciocalteu assay (r=0.81 and 0.80, respectively; **Table S4**). ABTS could be considered one of the most common radical scavenging assays for wine evaluation, together with DPPH (1,1-diphenyl-2-picrylhydrazyl radical), and the results provided by these two methods show a strong positive correlation (r=0.949) (Floegel, Kim, Chung, Koo, & Chun, 2011). Previous studies indicated that the most important variables contributing to the wines' antioxidant activity (in those cases measured through DPPH methods) were the contents of total polyphenols, total flavanols (Arnous, Makris, & Kefalas, 2002), total flavonoids, and proanthocyanidin indices together with several individual phenolic compounds (Cassino, Gianotti, Bonello, Tsolakis, Cravero, & Osella, 2016). It is estimated that fifty percent of the total red wines scavenging radical activity (ABTS, DPPH, and other methods) was attributed to polymeric phenolic compounds (Fernández-Pachón, Villaño, García-Parrilla, & Troncoso, 2004), whereas presumably polymeric pigments are less important (Arnous et al., 2002; Travaglia, Bordiga, Locatelli, Coïsson, & Arlorio, 2011). Nevertheless, the occurrence of polymerization slightly increased the stoichiometry of the radical scavenging reaction when compared with the reaction of monomeric phenolics against the DPPH• radical (Ricci et al., 2016).

A comprehensive survey on polymeric proanthocyanidins (PAs) in skins and seeds of 37 *Vitis vinifera* L. cultivars showed that the antioxidant activity was negatively correlated (r= –0.61) with the PAs degree of polymerization assessed by the "modified" acetic acid-vanillin method (Travaglia et al., 2011). On the contrary, Fortes Gris, Mattivi, Ferreira, Vrhovsek, Curi Pedrosa, & Bordignon-Luiz (2011) found that the antioxidant activity measured as ABTS assay was directly correlated to the mean degree of polymerization (mDP) determined after acid-catalysis in the presence of excess phloroglucinol (i.e. phloroglucinolysis). Therefore, there is a need for further research to better understand the conditions that support the change in tannin size (i.e. increasing or decreasing).

4.2. Discriminant analysis and other statistical methods applied to phenolic indices and authenticity of red wines

The greater compositional differences between grape varieties rely on the berries' phenolic composition and especially on their flavan-3-ol contents (Santos-Buelga, Francia-Aricha, & Escribano-Bailón, 1995), which is already used to ascertain the origin and authenticity of grape varieties. For example, some comprehensive indexes, such as total phenolic compounds and total tannins in seeds, were useful tools to discriminate Cabernet Sauvignon and Merlot grape varieties from Bordeaux vineyards (Lorrain, Chira, & Teissedre, 2011), and wines from two clones of Cabernet Sauvignon as well (Burin, Freitas Costa, Rosier, & Bordignon-Luiz, 2011). Moreover, the phenolic composition and color characteristics were found to be key parameters to classify wines from grape varieties in Greece-Crete (Basalekou, Strataridaki, Pappas, Tarantilis, Kotseridis, & Kallithraka, 2016) and Turkey (Sen & Tokatli, 2014). A study conducted in a subtropical region of Brazil on red wines from Italian red varieties ('Ancellotta', 'Rebo', 'Nebbiolo', 'Barbera', and 'Teroldego') showed that the grape variety rather than vintage (2011 and 2012) exerted the predominant effect on the phenolic content and antioxidant activity of the wines (Sartor et al., 2017). Similarly, Heras-Roger et al. (2016) characterized 250 commercial Spanish red wines by means of 80 physico-chemical parameters and found that discriminant analysis (DA) was able to distinguish the wines according to the variety despite the large influences of winemaking techniques and vintage. Among the varieties considered, the DA showed five partially overlapping clusters, and according to the leave-one-out test about 90 % of all cases were correctly classified. This is in agreement with the effectiveness observed for DA in the present study to classify the analyzed wines according to the variety, regardless of the origin and the winemaking technique.

Concerning the evaluation of both variety and vintage effects, Pajović Šćepanović, Wendelin, Raičević, & Eder (2019) analyzed 43 wines from Montenegro and, using a PCA approach, were able to emphasize the effect of variety as more affecting than that of the vintage. Both vintage and variety were discriminated using color characteristics and phenolic data, all subjected to an orthogonal partial least square-discriminant analysis (OPLS-DA) statistical method, in the study by Sen & Tokatli (2016). The authors considered a sample set of 63 red wines, evidencing as the most influential UV-Vis spectral regions for classification purposes the portions between 414-458 nm and 514-538 nm.

Finally, another application of these techniques could be related to the geographical origin of wines, a useful factor for wine authenticity. Peña-Neira, Hernández, García-Vallejo, Estrella, & Suarez (2000) were able to distinguish Spanish wines according to their geographical origin from phenolic compounds data.

4.3. Phenolic diversity of main Italian monovarietal red wines

The level of grape ripening and the winemaking process play a pivotal role in the determination of the characteristics of wine phenolics (Mattivi et al., 2006; Mattivi et al., 2009; Unterkofler et al., 2020). In the present study, it was possible to characterize the most important monovarietal Italian wines from different regions by a wide range of compositional and color parameters, which are affected by the general practices used in each production zone for the elaboration of these wines. This led to a specific characterization of the main Italian red wines by the combination of the varietal traits and the usual winemaking procedures adopted in each production zone.

The eleven varieties studied showed a conspicuous variability for all the tested parameters. This is particularly evident for the compounds representing the main focus of this study, namely phenolics, which determine the aging aptitude and important sensory perceptions such as color, astringency, and bitterness (Ma et al., 2014; Paissoni et al., 2018). Both intra- and inter-variability among monovarietal wine groups was found also in a similar study carried out on ten international and Spanish varieties (Heras-Roger et al., 2016). This double effect hinders the ability to provide distinct ranges (e.g. by significance tests) for each monovarietal wine and parameter combination, allowing only for an indication of possible suitable groups. This particular behavior is clearly visible in the plot in **Figure 4a**, which highlights the presence in the obtained dataset of hypothetical groups of wines based on proanthocyanidins and vanillin assays. The monovarietal red wines are ordered as following, from the lowest to the highest average values: 'Corvina' – 'Teroldego', 'Cannonau', 'Montepulciano' – 'Primitivo' – 'Sangiovese' (Romagna and Toscana), 'Nerello Mascalese' (limited number of samples), 'Raboso Piave' – 'Aglianico' – 'Nebbiolo' – 'Sagrantino'. The combination of these assays aims to represent the richness of flavanols together with their indicative degree of polymerization and possible reactivity, and therefore this ordering could give an indication of the sensory properties related to tannins. However, chemical analyses are not sufficient to fully describe wine bitterness and astringency, and specific sensorial studies were conducted on these wines to achieve this aim (Piombino et al., 2020).

To fulfil the same hypothetical grouping previously performed for flavanols, **Figure 4b** allows to extrapolate the following groups according to total and monomeric anthocyanin contents: 'Corvina', 'Nebbiolo' – 'Nerello Mascalese' (which however was represented by a limited number of samples showing broad distribution) – 'Sagrantino', 'Primitivo', 'Montepulciano' – 'Cannonau', 'Sangiovese' (Toscana and Romagna) – 'Raboso Piave', 'Aglianico' – 'Teroldego'. The different distribution of these two obtained sets (**Figures 4a-4b)** permits to draw some trends. Some wines, such as ‘Corvina’, ‘Primitivo’, ‘Sangiovese’, ‘Raboso Piave’, and ‘Aglianico’, maintain a certain correspondence in these two sets. Three wines, namely ‘Cannonau’, ‘Montepulciano’, and ‘Teroldego’, were grouped together for a moderate flavanol composition but performed well for anthocyanin contents, and in particular ‘Teroldego’ showed the highest values. Finally, the remaining varieties evidenced a high flavanols content not tied to that of anthocyanins: this is the case of ‘Nebbiolo’ and ‘Nerello Mascalese’, that showed low anthocyanin richness, and of ‘Sagrantino’, which was characterized by a medium total anthocyanin content but a low monomeric percentage.

Regarding anthocyanin content, it is noteworthy that ‘Corvina’, ‘Nebbiolo’, and two samples of ‘Nerello Mascalese’ were previously found to share the same plot region in the **Figure 4b** visualization, well below the other wines tested. These varieties are characterized by a low-to-medium content of anthocyanins in grape skins and by a particular grape anthocyanin profile, evidencing a relative high prevalence in di-substituted anthocyanin forms, mainly peonidin and cyanidin, which are easily oxidable in the first phases of winemaking (Mattivi et al. 2006; Nicolosi Asmundo, Arena, & Grasso, 2007). These aspects could provide a further interpretation of the anthocyanin results for these wines.

4.3.1. The case of ‘Sangiovese’ wines and their production zones

‘Sangiovese’, the most planted Italian grape variety and among the top 10 red varieties for worldwide planted surface (Anderson & Aryal, 2013), was represented in this study by wines originating from two different production regions, i.e. Emilia-Romagna (n=12) and Toscana (n=7). In general, the main compositional data (**Figure 2**; **Table S1**) show similar (p>0.05) values for both zones.

Interestingly, also the data on phenolic compounds (**Figure 3**; **Table S3**) evidenced similar average values and did not lead to significant differences (p>0.05). Total phenols, determined through the

Folin-Ciocalteu assay, was the parameter leading to the highest (but not significant; $p>0.05$) differences, resulting in an increase of about 13 % for the Romagna samples when compared to those from Toscana. Even if not significant, this higher content of total phenols could have a sensory impact and likely be related to the significantly higher harsh character discriminating the astringency profile of 'Sangiovese' wines from Romagna compared to samples from Toscana (Piombino et al . 2020). However, when the phenolic composition was assessed by more specific indexes, such as the proanthocyanidins and vanillin assays or total anthocyanins index, the two considered 'Sangiovese' regions achieved very similar results (3.1, 1.2, and 11.0 % difference on average values, respectively) and their samples were positioned very closely according to these parameters (**Figure 4**). Despite the lack of significant ($p>0.05$) differences among the main parameters analyzed, mainly due to the high variability of the wines analyzed, a multivariate approach was a viable way to discriminate the two zones considered, but only to a limited extent (**Table 1**). This aspect paves the way for the feasibility of applying these tools also for zone discrimination, but for this purpose more specific studies are needed.

5. CONCLUSIONS

In this study, 110 samples of selected Italian monovarietal red wines from a single vintage were characterized by different analytical methods aimed to determine their phenolic composition, antioxidant capacity, color characteristics, and basic enological parameters. This large dataset, both in terms of samples and parameters determined, allowed to provide an insightful reference for the composition of the main Italian monovarietal red wines and made possible to compare and relate different analytical protocols. The determined parameters were shown to be useful in the discrimination of wines using simple and rapid analytical methods. The results clearly highlighted that Italian grape varieties can be traced in red wines by means of several variables, including the type and content of extractable phenolic compounds. Further implications of this dataset on the variety-related composition for main phenolic classes can be related to a range of production variables, such as grape ripening and winemaking techniques. Given that the vintage variability affects the phenolic content, further studies could be focused on the wine phenolic profile, a parameter that is mainly affected by the grape cultivar. The extension of this study should enhance these conclusions, providing also additional results to be related to genetic studies aimed to the elucidation of the phenolic metabolism in grapes.

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Table 1. Confusion matrix for the DA cross-validation of Italian red wines (except 'Nerello Mascalese') with an overall correctly classified value of 61.7 %.

from \ to	1	2	3	4	6	7	8	9	10	11	12	Total	Correct (%)
1	**3**	3	0	0	0	0	1	0	0	0	0	7	42.9 %
2	1	**8**	0	1	0	0	2	0	0	0	0	12	66.7 %
3	0	0	**11**	0	0	0	0	0	0	0	0	11	100.0 %
4	0	1	0	**4**	0	4	0	0	1	0	0	10	40.0 %
6	0	1	0	1	**5**	3	0	0	0	0	1	11	45.4 %
7	1	2	0	4	1	**2**	0	0	0	0	0	10	20.0 %
8	0	5	0	1	1	0	**2**	0	0	0	0	9	22.2 %
9	0	0	0	0	0	0	0	**10**	0	1	0	11	90.9 %
10	0	0	0	0	1	0	0	0	**9**	0	0	10	90.0 %
11	0	0	0	0	0	0	1	1	0	**6**	1	9	66.7 %
12	0	0	0	0	0	0	0	0	0	1	**6**	7	85.7 %
Total	5	20	11	11	8	9	6	11	10	8	8	107	61.7 %

Legend of red wines: (1) 'Sangiovese' Toscana; (2) 'Sangiovese' Romagna; (3) 'Nebbiolo'; (4) 'Aglianico'; (6) 'Primitivo'; (7) 'Raboso Piave'; (8) 'Cannonau'; (9) 'Teroldego'; (10) 'Sagrantino'; (11) 'Montepulciano'; (12) 'Corvina'.

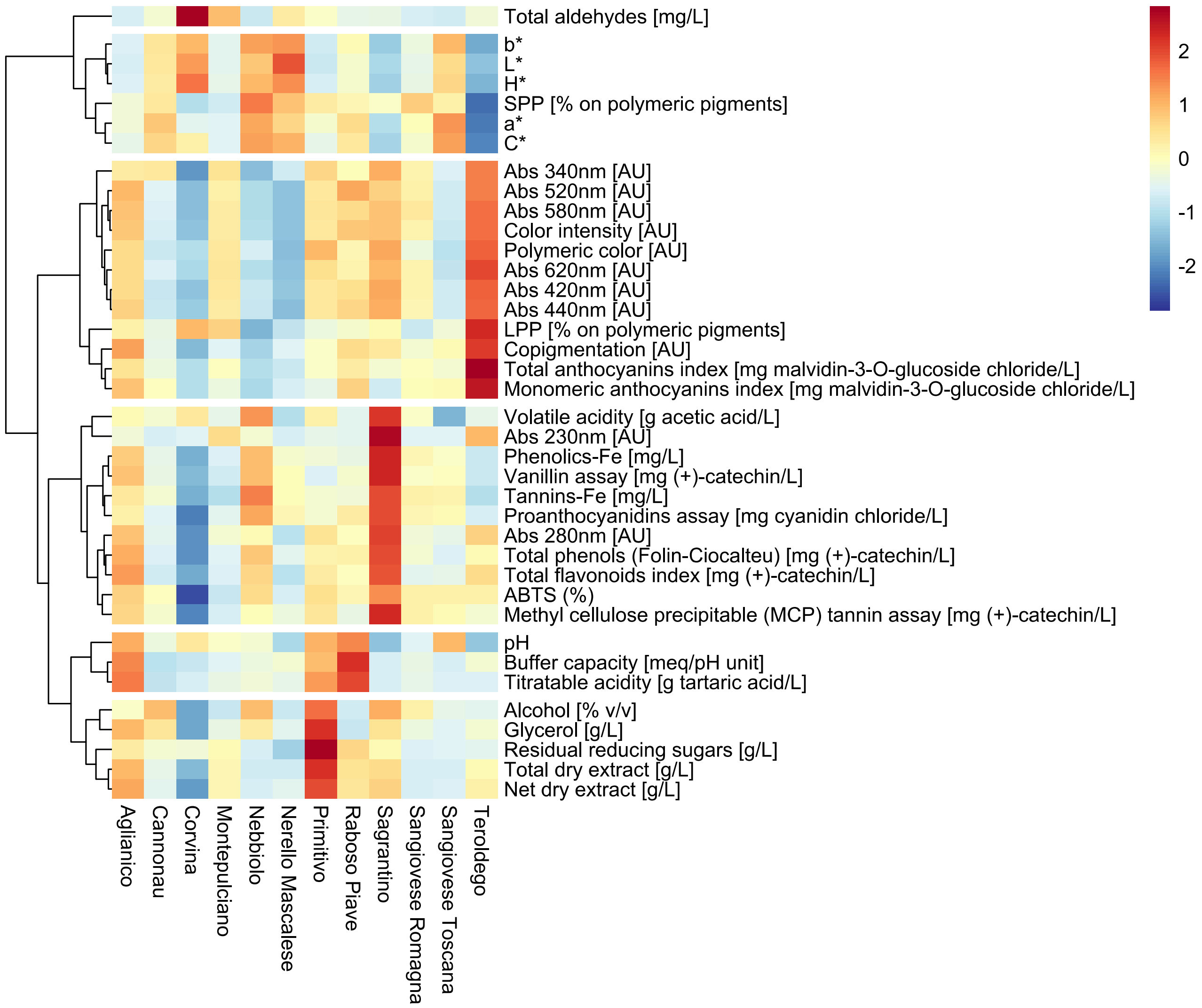

Figure 1. Heat map visualization of the monovarietal red wines according to the determined parameters classified by a hierarchical cluster.

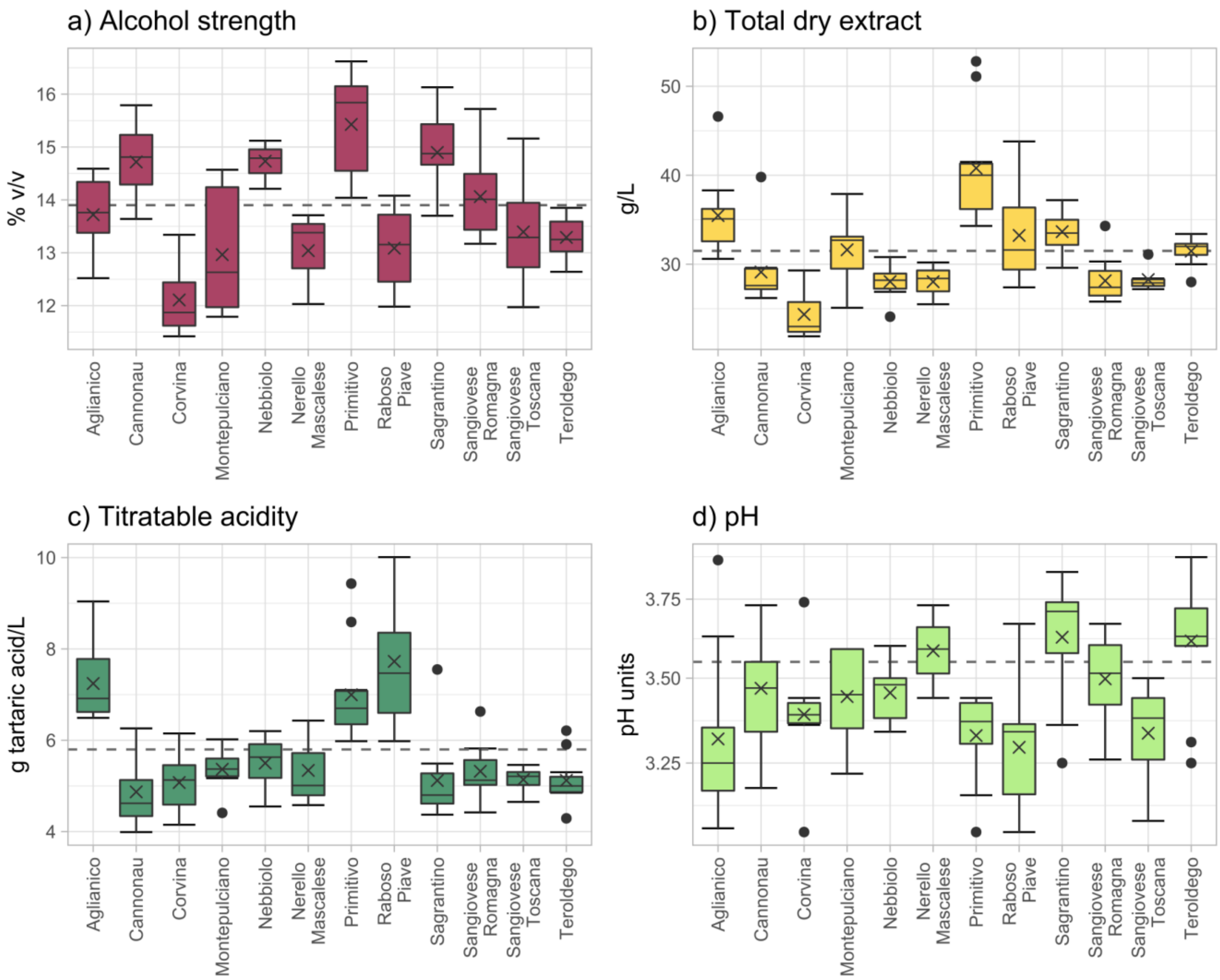

Figure 2. Variability of the main base compositional parameters as grouped by wine: alcohol by volume (a), dry total extract (b), titratable acidity (c), and pH (d). For each graph, the dashed line represents the average value of the whole dataset, while the multiplication sign (×) points the average value in each wine group.

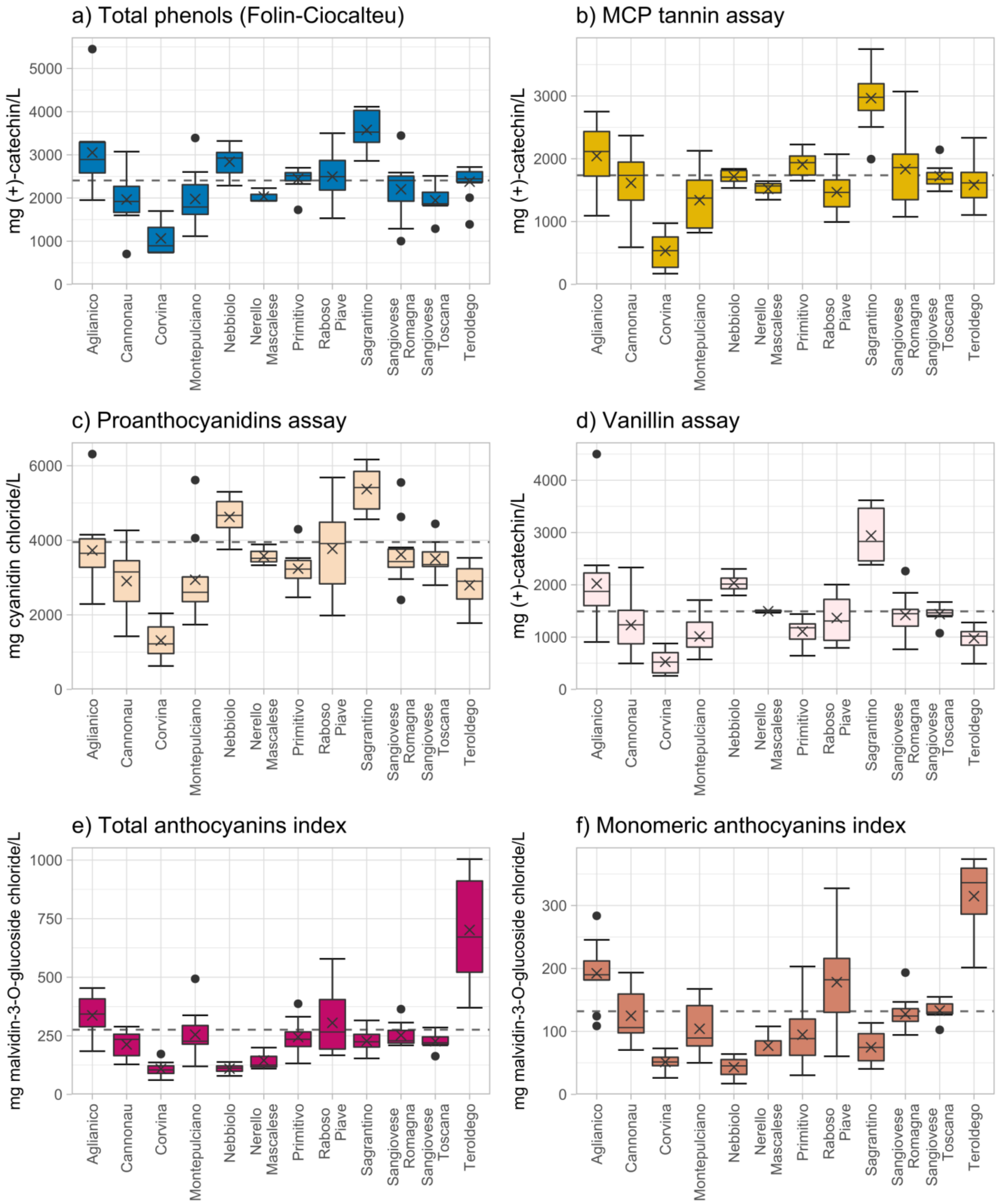

Figure 3. Variability of the phenolic parameters as grouped by wine: total phenols (Folin-Ciocalteu assay; a), methyl cellulose precipitable tannins (MCP; b), proanthocyanidins assay (PROC; c), vanillin assay (VAN; d), total anthocyanins index (e), and monomeric anthocyanins index (f). For each graph, the dashed line represents the average value of the whole dataset, while the multiplication sign (×) points the average value in each wine group.

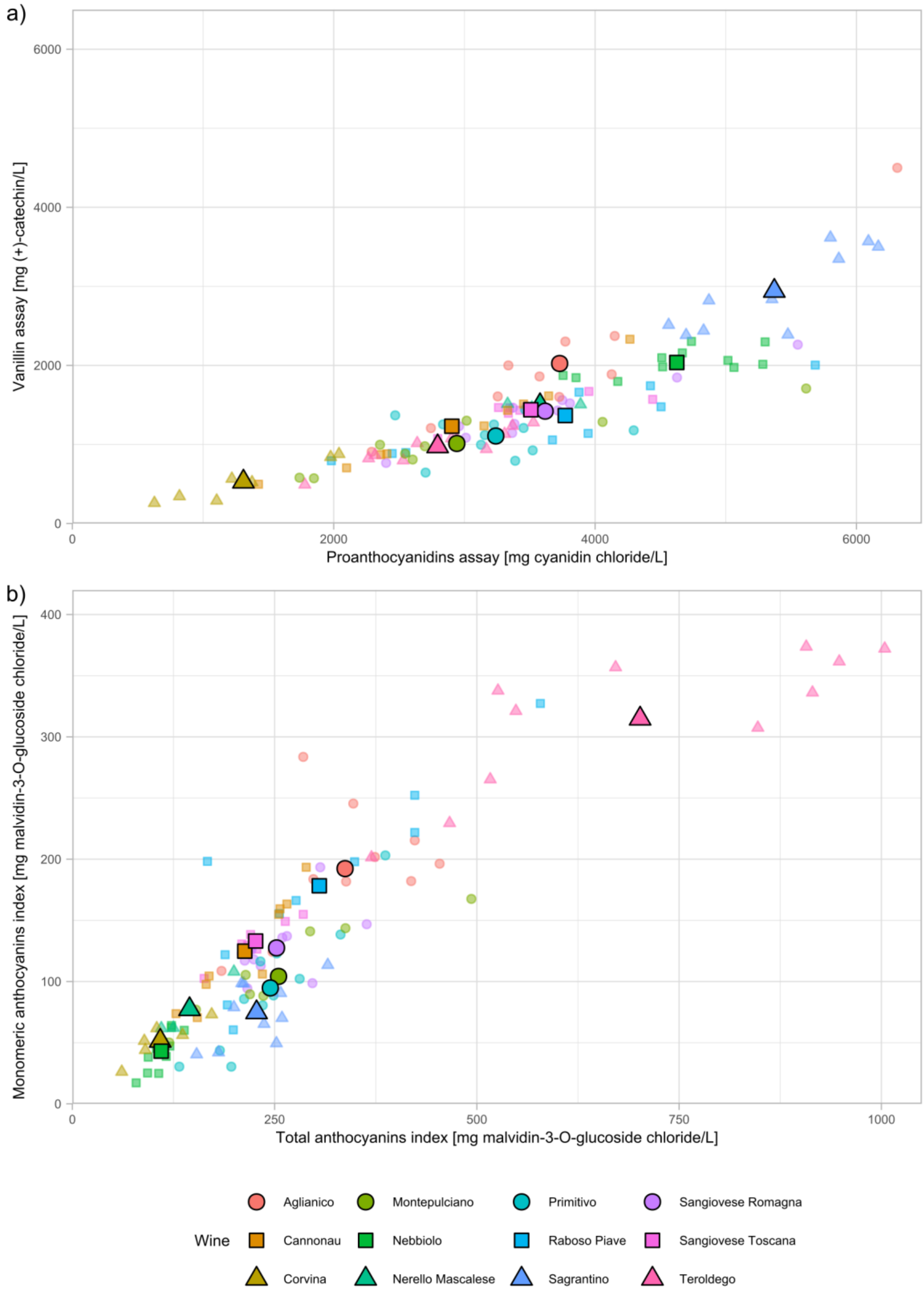

Figure 4. Proanthocyanidins assay (PROC) *vs* vanillin assay (VAN) scatter plot (a), and total *vs* monomeric anthocyanins scatter plot (b) for the 110 monovarietal wines tested, grouped by wine denomination and region. For both panels, small points indicate each observation (N=110), while big points (black-bordered) represent the average value for each wine group.

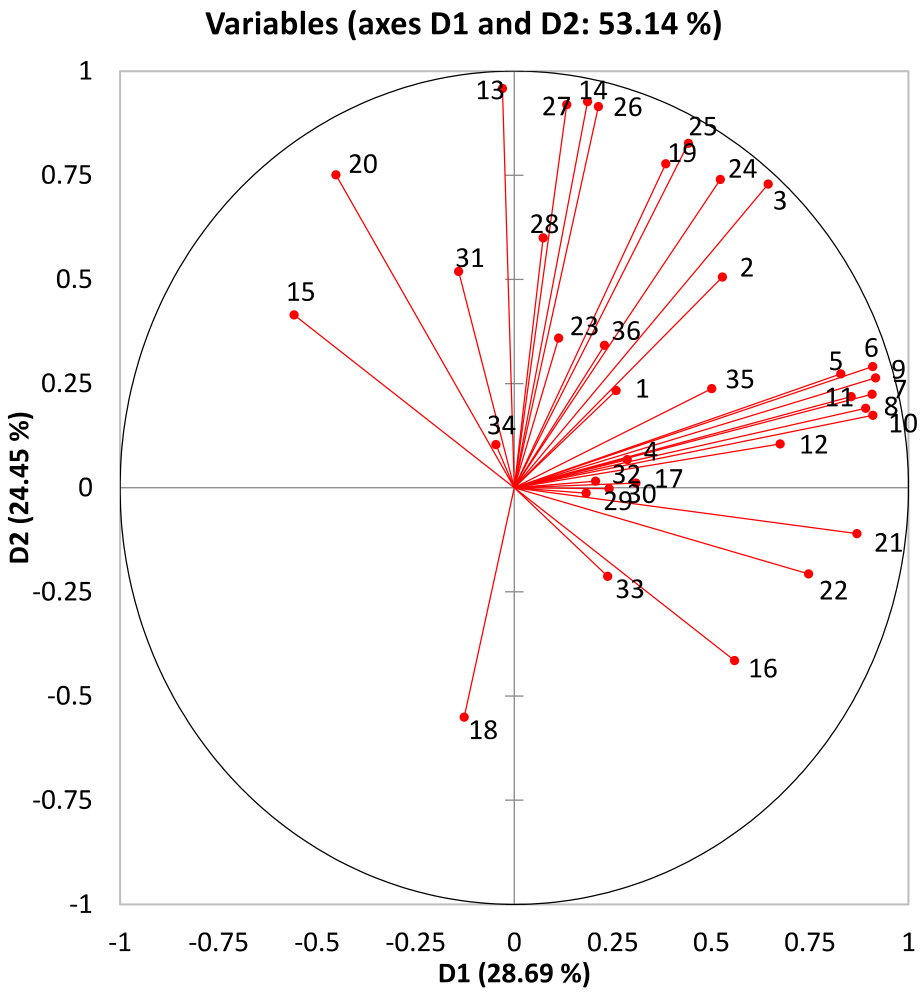

Figure 5: PCA plot of 36 physico-chemical parameters of Italian monovarietal red wines. Legend: (1) pH; (2) 230 nm; (3) 280 nm; (4) 230 nm/280 nm; (5) 340 nm; (6) 420 nm; (7) 440 nm; (8) 520 nm; (9) 580 nm; (10) 620 nm; (11) Polymeric color; (12) Copigmentation; (13) Tannins-Fe; (14) Phenolics-Fe; (15) SPP; (16) LPP; (17) Buffer capacity; (18) Total aldehydes; (19) ABTS; (20) Tannin-Fe/Anthocyanin ratio; (21) Total anthocyanins; (22) Monomeric anthocyanins; (23) Total/monomeric anthocyanins; (24) Total flavonoids; (25) Total phenols (Folin-Ciocalteu); (26) Proanthocyanidins assay; (27) Vanillin assay; (28) Alcohol; (29) Residual reducing sugars; (30) Titratable acidity; (31) Volatile acidity; (32) Malic acid; (33) Lactic acid; (34) Tartaric acid; (35) Total dry extract; (36) Glycerol.

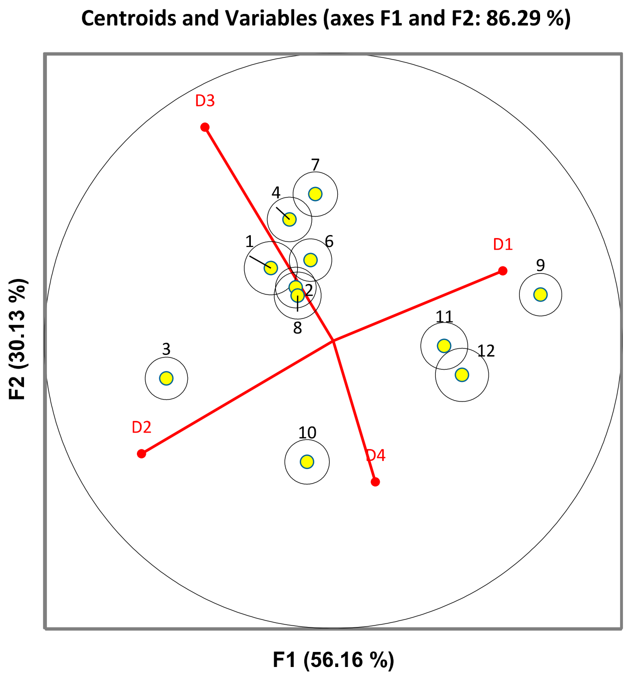

Figure 6. LDA plot of Italian monovarietal red wines with centroid of sample groups and variables (i.e. $PC_{1\text{-}4}$ scores). Legend: (1) 'Sangiovese' Toscana; (2) 'Sangiovese' Romagna; (3) 'Nebbiolo'; (4) 'Aglianico'; (6) 'Primitivo'; (7) 'Raboso Piave'; (8) 'Cannonau'; (9) 'Teroldego'; (10) 'Sagrantino'; (11) 'Montepulciano'; (12) 'Corvina'.