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Research Article

Antioxidant Composition of a Selection of Italian Red Wines and Their Corresponding Free-Radical Scavenging Ability

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This study correlates the antioxidant composition profiles and the overall antioxidant capacities of 36 Italian red wine samples. The samples were fully characterized by chromatographic and spectrophotometric techniques. The overall antioxidant capacity was determined by titrating a solution of the semistable free radical DPPH (1,1-diphenyl-2-picrylhydrazyl) with each wine sample followed by Electron Paramagnetic Resonance (EPR) spectroscopy and then measuring the resulting decrease in DPPH-signal. The antioxidant activities of the samples were expressed as (+)-catechin equivalents and related to their antioxidant composition profiles. Samples with a high polyphenol content showed a high DPPH scavenging ability as well. Seven well-defined groups, mainly constituted by wines coming from the same cultivar, were evidenced by PCA analysis. Alcohol content and pH did not influence the wine DPPH scavenging ability. The most important variables contributing to the wines' antioxidant power are total flavonoid, total phenol, and proanthocyanidin indices together with fertaric acid, *trans*-caftaric acid, *trans*-coutaric acid, and both quercetin glucoside and quercetin glucuronide. EPR is demonstrated to be faster than the other analytical methods (spectrophotometric and chromatographic analyses) to determine the wine overall antioxidant activity.

1. Introduction

Wine has been part of human culture for thousands of years and is an important component of the traditional Mediterranean diet. Although the excessive use of alcohol can lead to serious health problems, a number of studies suggest that moderate consumption of wine (especially red) may provide health benefits. According to the *Dietary Guidelines for Americans* [1], moderate alcohol consumption is defined as having up to 1 drink (a glass, 150 mL 12% alcohol wine) per day for women and up to 2 drinks (two glasses, 300 mL 12% alcohol wine) per day for men. Positive effects derive from the strong antioxidant properties of polyphenolic compounds [2]. Red wines generally contain higher levels of polyphenolics than white ones. These compounds not only contribute to the colour, flavour, astringency, and bitterness of a wine but also serve to fight free radicals in the body that cause disease and ageing. Epidemiological studies pointed out that the consumption of red wine has been shown to increase the body's antioxidant capacity and is associated with a lower risk of mortality from cardiovascular diseases [3, 4].

There are two major classes of wine phenolics: non-flavonoids and flavonoids. The relative amount and distribution of these compounds depend on a variety of factors such as grape variety, vineyard location, climate, soil type, cultivation practices, harvesting time, production process, and wine ageing [5–8].

The class of flavonoids includes several molecules characterized by their functionalities on the benzene rings. The most important flavonoids in wine are anthocyanidins, flavanols (also known as catechins or flavan-3-ols), and flavonols (including quercetin and myricetin). Proanthocyanidins,

dimers, or oligomers of catechin and epicatechin and their gallic acid esters are also classified as flavonoids. Red wines contain wide-ranging concentrations of proanthocyanidins.

The term nonflavonoid usually includes different classes of substituted phenols. These different congeners can be grouped as benzoic-based compounds (i.e., vanillic and gallic acids), benzaldehydes (vanillin and syringaldehyde), cinnamic acids (*p*-coumaric, ferulic, and caffeic acids), and cinnamaldehydes (coniferyl aldehyde and sinapylaldehyde). These classes can be further subdivided by the number and type of substituents present.

Ascorbic acid and sulfur dioxide, SO_2, also contribute to the antioxidant power of wine [9]. These compounds are naturally present in wine in very low amounts but are often added as preservatives during the winemaking process.

SO_2 exerts its antioxidant effect by destroying the polyphenol oxidase (PPO) enzyme responsible for phenol oxidation [10], but it was observed [11] that the content of polyphenols and flavonoids was similar in organic and conventional red wines as well as their antioxidant activity.

A wide variety of compounds contributes to the antioxidant power of wine, making it difficult to determine the relative contribution of each antioxidant species. Profound interactions between the compounds may take place, and the total antioxidant power of a wine may not correspond to the sum of the antioxidant capacities of each single molecule [12].

Recently, studies performed on grape skins, seeds [13–15], and wines [16–18] have appeared in the literature. These studies correlate the overall antioxidant power of the different wine samples (*via* EPR determination) with the total amount of polyphenols, but there is little information about the interactions within the single class of compounds.

To date, the antioxidant parameters of most wines produced in Italy have not been fully determined. There are only a few works regarding Italian wines [19], although one thorough study on wines coming from different zones of Campania (Italy) has been published [20]. This lack of available information is unfortunate, since, consequently, wine producers do not always take advantage of the commercial value associated with the antioxidant activity of wine, by making related claims in advertising.

From a statistical point of view, univariate methods have usually been applied for determining relationships between total or individual phenols with the antioxidant properties of red wines [21, 22]. This one-dimensional approach, however, fails to determine simultaneous correlations. To overcome this problem, the use of chemometric methods, which are intrinsically multivariate in nature, has been recognized as a valuable tool in wine science, for example, to assure wines' authenticity and quality [23], to classify their geographical origin [24, 25], and to evaluate their sensory properties [26].

In the present work, all the possible sources of antioxidant activity were sought to obtain information about their role in the overall antioxidant power.

Usually, the antioxidant activity of a molecule is measured by evaluating its ability to scavenge radicals produced in vitro. In this study, the moderately stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) organic radical was employed as a standard reagent for the measurement of the antioxidant capacity of wines. Even though this radical is not present in biological systems, it is often employed, since it is reasonably stable in water and in the presence of air [27]. Electron Paramagnetic Resonance (EPR) was the method of choice for providing this parameter [28].

The analyses were performed on red wines coming from different Italian regions, different cultivars, different winemaking techniques, and different ageing times, in order to have widely heterogeneous samples able to cover all possible factors influencing the antioxidant activity of grapes and wines [29]. All the data collected were analysed by the multivariate statistical method of the Principal Component Analysis (PCA) in order to find the possible correlation between the total antioxidant activity measured and the concentration of each class of antioxidant analysed.

2. Materials and Methods

2.1. Reagents. Ultrapure water, calcium oxide, phosphoric acid 85%, hydrogen peroxide, mixed indicator (pH 5.1), Folin-Ciocalteu reagent (phosphomolybdic acid and phosphowolframic acid mixture), absolute ethanol, hydrochloric acid 37%, sodium hydroxide solution 1 M, methanol (HPLC gradient grade), sulfuric acid 1 N, sodium carbonate, iron sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$), 1,1-diphenyl-2-picrylhydrazyl (DPPH), quercetin dehydrate, and *p*-coumaric acid were all purchased from Sigma-Aldrich (Milan, Italy) and used as received. Caffeic acid and ferulic acid were purchased from Extrasynthese (Lyon, France).

2.2. Samples. Thirty-six wines (Table 1) from different Italian regions were analysed. They were produced in experimental cellars, where the different steps of the winemaking process were controlled and traceable. The winemaking procedure used also allowed for limited and controlled addition of non-natural antioxidant products. The information about cultivars is shown in Table 1.

All the chemical and physical determinations were performed in triplicate.

Each sample was coded as indicated in Table 1. Numbers added at the end of each code identify repetitions of analysis.

2.3. Instrumentation. Determination of anthocyanins, flavonols, proanthocyanidins, and total phenols was carried out using a Jasco V-550 UV-VIS spectrophotometer (Tokyo, Japan).

The qualitative-quantitative analysis of HCTAs and flavonols was performed with HP 1090 Hewlett-Packard (Palo Alto, California, USA) HPLC liquid chromatograph equipped with a Diode Array Detector (DAD).

For EPR determination, a JEOL FA-200 X band EPR spectrometer (JEOL Ltd., Tokyo, Japan) with a flat cell (JEOL ES-LC11) was used.

2.4. Analytical Methods. The concentrations of alcohol (OH) and ascorbic acid (AA), pH, and free SO_2 content were determined according to the standard procedures reported in the EEC 2676/90.

Table 1: An overview of the wine samples investigated.

Cultivar	Code	PCA code	Region	Vintage	Winemaking technique
Albarossa	A10T	1T(1,2,3)	Piedmont	2010	Traditional
Albarossa	A10C	2C(1,2,3)	Piedmont	2010	With cryomaceration
Albarossa	A10MC	3MC(1,2,3)	Piedmont	2010	With hot prefermentative maceration
Cornarea	C10T	4T(1,2,3)	Piedmont	2010	Traditional
Cornarea	C10C	5C(1,2,3)	Piedmont	2010	With cryomaceration
Cornarea	C10MC	6MC(1,2,3)	Piedmont	2010	With hot prefermentative maceration
Refosco	R10Ve	7Ve(1,2,3)	Veneto	2010	Traditional
Refosco	R10Ma	8Ma(1,2,3)	The Marches	2010	Traditional
Refosco	R09Ve	9Ve(1,2,3)	Veneto	2009	Traditional
Refosco	R09Ma	10Ma(1,2,3)	The Marches	2009	Traditional
Nero d'Avola	NA10Ve	11Ve(1,2,3)	Veneto	2010	Traditional
Nero d'Avola	NA10Ma	12Ma(1,2,3)	The Marches	2010	Traditional
Nero d'Avola	NA09Ve	13Ve(1,2,3)	Veneto	2009	Traditional
Nero d'Avola	NA09Ma	14Ma(1,2,3)	The Marches	2009	Traditional
Primitivo	P08	15_(1,2,3)	Apulia	2008	Traditional with ageing in barriques
Primitivo	P09	16_(1,2,3)	Apulia	2009	Traditional with ageing in barriques
Primitivo	P10	17_(1,2,3)	Apulia	2010	Traditional with ageing in barriques
Cabernet s.	CaT101	18_(1,2,3)	Piedmont	2001	Vineyard with canopy management
Cabernet s.	CaT201	19_(1,2,3)	Piedmont	2001	Grapes with canopy management
Cabernet s.	CaT301	20_(1,2,3)	Piedmont	2001	Grapes with canopy management
Cabernet s.	CaT401	21_(1,2,3)	Piedmont	2001	Grapes with canopy management
Cabernet s.	CaT501	22_(1,2,3)	Piedmont	2001	Grapes with canopy management
Cabernet s.	CaT601	23_(1,2,3)	Piedmont	2001	Grapes with canopy management
Cabernet s.	CaT701	24_(1,2,3)	Piedmont	2001	Grapes with canopy management
Cabernet s.	CaT801	25_(1,2,3)	Piedmont	2001	Grapes with canopy management
Cabernet s.	CaT901	26_(1,2,3)	Piedmont	2001	Grapes with canopy management
Uvalino	U06	27_(1,2,3)	Piedmont	2006	Traditional
Uvalino	U04	28_(1,2,3)	Piedmont	2004	Traditional
Primitivo	P10M	29M(1,2,3)	Apulia	2010	Traditional with ageing in barriques
Primitivo	P10SM	30SM(1,2,3)	Apulia	2010	Traditional with ageing in barriques
Primitivo	P11M	31M(1,2,3)	Apulia	2011	Traditional with ageing in barriques
Primitivo	P11SM	32SM(1,2,3)	Apulia	2011	Traditional with ageing in barriques
Gaglioppo	G09nosd	33_(1,2,3)	Calabria	2009	Without seeds in steel
Gaglioppo	G09sd	34_(1,2,3)	Calabria	2009	With seeds in steel
Gaglioppo	G09nosdbq	35_(1,2,3)	Calabria	2009	Without seeds in barriques
Gaglioppo	G09sdbq	36_(1,2,3)	Calabria	2009	With seeds in barriques

The determination of total phenol (TP), total anthocyanin (TA), proanthocyanidin (PA), and total flavonoid (TF) indices was performed according to the methods described by Di Stefano et al. [30].

For hydroxycinnamoyl-tartaric acids (HCTAs) and flavonol determinations, chromatographic methods previously reported by Di Stefano and Cravero [31] were used.

HPLC separations were performed using a C18 column ODS Hypersil RP-18 (200 × 2.1 mm, 5 mm) (Thermo Scientific) and the mobile phase gradient reported in Table 2. Solvent A is $1 * 10^{-3}$ M phosphoric acid and solvent B is methanol. The flow rate was 0.25 mL min^{-1} and the volume injected was 20 μL.

Flavonols were determined registering the chromatogram at 360 nm [32]. In particular, quercetin glucoside (Q1), quercetin glucuronide (Q2), and myricetin (MY) were identified and quantified by the response factor method and reported as an equivalent of quercetin dehydrate.

HCTAs were identified and quantified by the chromatograms registered at 320 nm. The amount of HCTAs was reported as *p*-coumaric acid equivalents for *cis*- (CC) and *trans*- (TC) *p*-coumaroyl tartaric acid (*cis*-, *trans*-coutaric acid), as caffeic acid equivalents for *cis*- (CF) and *trans*- (TE) caffeoyl tartaric acids (*cis*-, *trans*-caftaric acid), and as ferulic acid equivalents for *trans*- (TL) feruloyl tartaric acid (*trans*-fertaric acid), using the response factor method.

Table 2: HPLC mobile phase gradient conditions.

Minutes	Solvent A H_3PO_4 0.001 M	Solvent B Methanol
0–5	From 95% to 90%	From 5% to 10%
5–20	From 90% to 70%	From 10% to 30%
20–30	From 70% to 40%	From 30% to 60%
30–40	From 40% to 0%	From 60% to 100%
40–45	0%	100%
45–50	From 0 to 95%	from 100 to 5%
50–55	Equilibration time	

The concentration of *cis*-feruloyl tartaric acid was too low to be determined.

2.5. EPR Measurements. For experiments of free-radical scavenging, 990 μL of a 250 μM DPPH solution in methanol was added to 10 μL of each wine sample. The mixture was then transferred to a flat cell for the analysis of the residual DPPH radicals. The measurements were performed 5, 10, and 15 minutes after the addition of DPPH. The signal areas were evaluated by double integrating the recorded EPR signal in the region between 326 and 330 mT. EPR spectroscopic analyses were carried out under the following conditions: temperature 25°C; magnetic field 329±5 mT; field modulation width 0.1 mT; field modulation frequency 100 KHz; receiver gain 200; time constant 0.03 s; sweep time 30 s; microwave power 4 mW. The percentage ratio of the signal areas of wine and reference samples after 5, 10, and 15 minutes from DPPH addition was calculated. The reference sample was prepared adding 10 μL of a 12% v/v ethanol aqueous solution to 990 μL of the 250 μM DPPH stock solutions in methanol. (+)-Catechin was chosen as the reference antioxidant to express the free-radical scavenging activity measured by EPR. Different amounts of a 400 μM (+)-catechin solution in methanol were added to 400 μL of a 250 μM DPPH methanol solution. Methanol was then added to reach the final volume of 500 μL. The obtained samples were 200 μM in DPPH and had a (+)-catechin concentration ranging from 8 to 56 μM. The percentage ratio of the signal areas of (+)-catechin and reference samples after 5, 10, and 15 minutes from DPPH addition was calculated. For (+)-catechin, the reference sample was prepared by adding 100 μL of methanol to 400 μL of the 250 μM DPPH stock solution in methanol. Figure 1 shows the antiradical activity observed for (+)-catechin.

The free-radical scavenging activity of the wines under investigation (T5, T10, and T15) was reported as the equivalent amount of (+)-catechin (mg L^{-1}) necessary to identically quench the EPR signal of DPPH after the same contact time.

2.6. Statistical Analysis. PCA is a pattern recognition method representing objects in a new reference system characterized by variables called Principal Components (PCs) well described elsewhere [33]. Briefly, PCs are orthogonal to each other and are computed hierarchically (the information accounted for by successive PCs is decreasing). Each PC has the property of explaining the maximum possible amount of variance contained in the original dataset. The PCs, which are expressed as linear combinations of the original variables, are used for an effective representation of the system under investigation with a lower number of variables than in the original case. The coordinates of the samples in the new reference system are called scores, while the coefficients of the linear combination describing each PC, that is, the weights of the original variables on each PC, are called loadings.

Principal Component Analysis and all graphical representations were performed using Statistica 7.1 (Dell Statistica, Tulsa, Oklahoma, USA) and Excel 2013 (Microsoft Corporation, Redmond, Washington, USA).

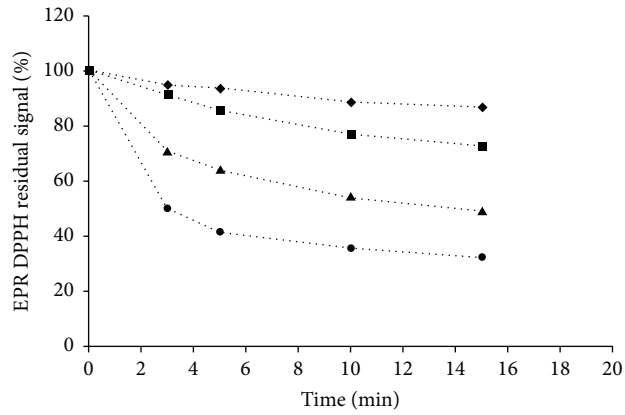

Figure 1: Quenching of radical activity by (+)-catechin solutions with time. 8 μM (diamonds), 24 μM (squares), 40 μM (up triangles), and 56 μM (circles).

3. Results and Discussion

Thirty-six wines obtained from the 9 cultivars (Table 1) were analysed for the 20 parameters reported in the experimental section. The wines were produced in experimental cellars where the use of chemical additives such as ascorbic acid was avoided, while the SO_2 addition was kept as low as possible.

Samples were produced using grapes grown in different areas of Italy (with a variety of soils and climatic characteristics) and from different cultivars. In this way, the set of samples guarantees adequate heterogeneity of the agronomic and viticulture characteristics.

The entire dataset, obtained analysing each wine sample, is available as supplementary material (in Supplementary Material available online at http://dx.doi.org/10.1155/2016/4565391). As expected, since the wines chosen for the study are very heterogeneous, the experimental values obtained for antioxidant concentrations are spread over a wide range.

In particular, the total phenol index ranged from 765 to 5745 mg L^{-1} (the average being 2160 mg L^{-1}) [34, 35], the total anthocyanin index from 18 to 687 mg L^{-1}, the total flavonoid index from 810 to 5260 mg L^{-1}, and the proanthocyanidin index from 761 to 6900 mg L^{-1}. The alcohol content ranged between 9.64 and 16.38% v/v. It is important to point out that the majority of SO_2 comes from the addition of potassium metabisulfite to grapes and wine. The wines studied here were

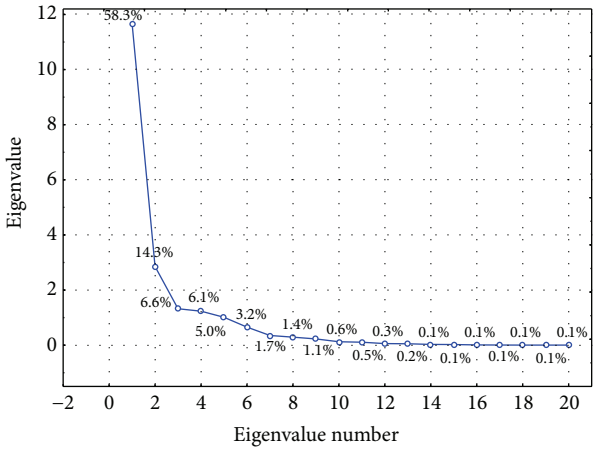

Figure 2: Scree plot obtained by performing PCA on the complete dataset.

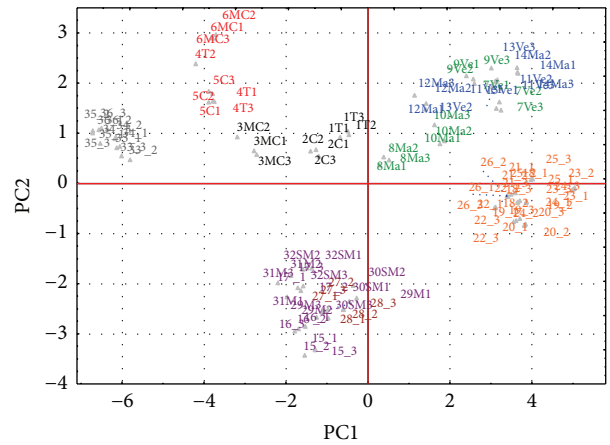

Figure 3: Score plot of PC1 versus PC2.

characterised by very low free SO_2 values ranging from 0 to 28 mg L^{-1}. These analytical results confirmed the claims of cellars who added only the minimum SO_2 amount required to preserve the wine, even if higher amounts are allowed by the Italian law. The ascorbic acid concentration was below the detection limit for all the samples measured.

HCTAs (caftaric, coutaric, and fertaric acid) and flavonols (quercetin and myricetin) were present in low concentrations, as expected [36]; even if they were originally present at high concentration in grapes, their amount decreased significantly during fermentation and ageing.

The quenching of DPPH signal is a function of both contact time and the amount of antioxidant compounds added. Figure S1, reported as an example, shows the decrease of the DPPH EPR signal with time after the addition of a given amount of wine. The most common standard employed to quantify the overall antioxidant capacity of wine is (+)-catechin. Noteworthy, kinetics of free-radical quenching of the wines under investigation and (+)-catechin were very similar during the EPR experiment, giving rise to the meaningful T5, T10, and T15 parameters as described in Section 2.5.

Gaglioppo and Cornarea wines showed the highest antioxidant activity, while Nero d'Avola and Cabernet showed the lowest one. For the Cabernet wines (produced in 2001), this fact can be explained by the ageing effect, which leads to a drastic depletion of the polyphenol content. In general, samples with a high polyphenol content showed a high DPPH scavenging ability as well.

The experimental data clearly indicate profound differences among the samples, and finding correlation in the dataset appears to be a complex task especially by the classical univariate approach. Therefore, PCA analysis was performed on the complete dataset in which the variables were autoscaled to eliminate their inhomogeneity. The scree plot in Figure 2 shows that the first PC (PC1) explains about 61% and the second PC (PC2) about 15% of the total variance contained in the original dataset. Therefore, the successive PCs can be considered as not statistically significant.

In Figure 3, the score plot obtained for the first two PCs is reported in which the samples of the same cultivar have the same colour code. The first two PCs allow effective separation of the samples into different classes. Results from three sample replicates proved to be very similar, indicating excellent repeatability for all parameters examined.

In particular, seven well-defined groups can be identified (Figure 3), mainly constituted by wines coming from the same cultivar. The exceptions are Nero d'Avola and Refosco, whose data partially overlapped.

Accurate interpretation of the parameters leading to this grouping of the samples requires the loading plot information reported in the histogram of Figure 4 that represents the weights of the original variables on each PC.

Many of the variables studied show negative weights on PC1. In particular, the variables related to the EPR analyses and the proanthocyanidin, the total flavonoid, and the total phenol indices are located at high negative weights. Moreover, a lower but still significant contribution is also present for quercetin glucoside, quercetin glucuronide, and *trans*-caftaric acid, which have a lower negative weight. pH is the only variable placed at a positive weight on PC1, but its value is not so high.

Within the first PC, the EPR values and the content of polyphenols have the same sign, indicating that there is a strong correlation between them and that they account for the same information. For those reasons, the meaning of "antioxidant power" can be assigned to the macrovariables PC1. This macrovariable distinguishes very well between Gaglioppo and Cornarea (which have a high antioxidant capacity) from Cabernet sauvignon, Refosco, and Nero d'Avola. Figure 3 clearly illustrates the antioxidant activity attribute of the cultivars under study, showing their position along the PC1 axis. Antioxidant power increases, in fact, when moving from the right to the left side of the figure. In detail, the samples placed in the negative part of the PC1 are characterized by high values of the variables having negative weight on this PC and by low values of the ones having positive weight. The opposite is true for the samples placed in the positive part of PC1.

It is important to note that the antioxidant power of the Gaglioppo samples is much stronger than that of all the other

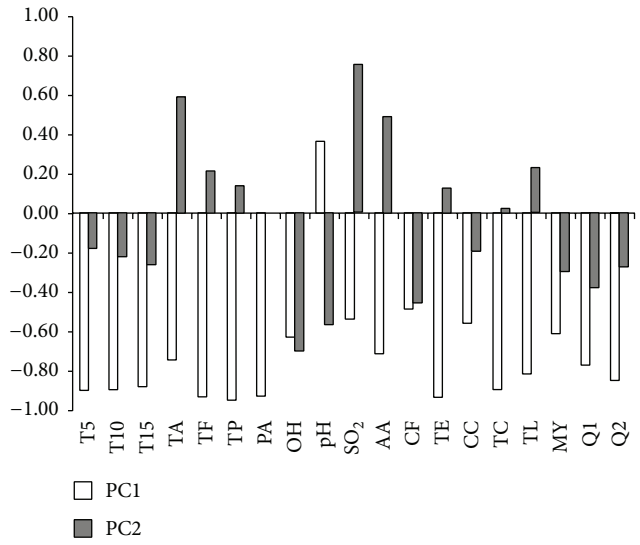

Figure 4: Loading plot reporting PC1 versus PC2. The following abbreviations were used: T5, T10, and T15: wine free-radical scavenging activity after 5, 10, and 15 minutes, TA: total anthocyanin index, TF: total flavonoid index, TP: total phenol index, PA: proanthocyanidin index, OH: ethanol content (%vol.), pH: wine pH value, SO_2: free SO_2 content, AA: ascorbic acid concentration, CF: *cis*-caffeoyl tartaric acid concentration, TE: *trans*-caffeoyl tartaric acid concentration, CC: *cis*-*p*-coumaroyl tartaric acid concentration, TC: *trans*-*p*-coumaroyl tartaric acid concentration, TL: *trans*-feruloyl tartaric acid concentrations, MY: myricetin concentration, Q1: quercetin glucoside concentration, and Q2: quercetin glucuronide concentration.

wines, regardless of the different winemaking techniques used to produce them.

To further describe the differences among the abovementioned groups, the variables that constitute PC2 also have to be considered. PC2 describes a residual percentage of variance of about 15% (Figure 4) and it is mainly characterized by alcohol and pH, located at negative weights, and by free SO_2 and total anthocyanin index located at a positive weight.

In Figure 3, it is possible to see that PC2 separates the groups of Refosco, Nero d'Avola, Albarossa, and Cornarea samples (located at the top of the figure) from the group of Primitivo and Uvalino (located at the bottom).

The role of alcohol content and pH value on the DPPH-EPR assay was investigated in more detail. A sample of a wine characterized by very low alcohol content (9.5% v/v ethanol) was tested for DPPH scavenging activity and then its alcohol content was increased up to 12.00% and 14.50% v/v by adding absolute ethanol. No differences were observed on the DPPH scavenging activity before and after such ethanol additions. The effect of pH was evaluated by varying the pH of a natural wine from 2.98 to 3.98 through the addition of hydrochloric acid or sodium hydroxide, respectively. No variations in DPPH scavenging ability were observed. These experiments ruled out any direct effect of these parameters. The correlation observed on PC2 between ethanol content and the pH value can be explained considering that a high alcohol content in the wine samples derives from the use of grapes with a high sugar content (such as very mature grapes); this leads to a final product with a relatively high pH (low acidity). Indeed, Primitivo and Uvalino are both wines with a high alcohol content and low acidity, since they were made with very ripe grapes.

Despite the low amounts of SO_2 found in all the samples, this variable allows PC2 to highlight differences among the biological samples Primitivo and Uvalino with respect to Refosco, Nero d'Avola, Albarossa, and Cornarea that stem from microwinemaking processes. In the latter winemaking procedure, slightly more SO_2 is required to prevent oxidation, because of the small volume of grapes used.

4. Conclusions

In this work, 36 wine samples were fully characterised by chromatographic and spectrophotometric techniques, and their antioxidant activities were evaluated by DPPH-EPR assay. The EPR measure is quite fast and does not require any sample pretreatment.

The resulting dataset was subjected to multivariate PCA analysis. The loading plot shows that the most important variables contributing to the wines' antioxidant power are total flavonoid, total phenol, and proanthocyanidin indices together with fertaric acid, *trans*-caftaric acid, *trans*-coutaric acid, and both quercetin glucoside and quercetin glucuronide. From the score plot, it is possible to observe that PC1 can distinguish the seven different cultivars on the basis of their antioxidant capacity so that the meaning of "antioxidant power" can be assigned to the macrovariable PC1. On the other hand, PC2 is able to point out some differences among the samples arising from variations in pH, alcohol, and free SO_2. The observed differences among samples could not be evidenced with the classical univariate approach.

Additional Points

The authors studied 36 wines produced with grapes from different cultivars and areas of Italy. Red wines were assessed using spectrophotometric and HPLC determinations. Antioxidant power of red wine was tested with DPPH assays.

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

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