PAPER

ENOLOGICAL ELIGIBILITY OF GRAPE CLONES BASED ON THE SIMCA METHOD: THE CASE OF THE SANGIOVESE CULTIVAR FROM TUSCANY

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ABSTRACT

Sangiovese is the most widespread Italian red grape cultivar and it constitutes the basis of internationally known wines. It has a large diversity of clones whose performances vary with environmental conditions due to interaction with the weather and soil. In this study, the performance of grapes from seven Sangiovese clones was evaluated by analyzing grapes from four vineyards in the Chianti Classico Region in Tuscany over the ripening period. In order to assess the enological eligibility of grape clones, a grape reference model was developed using chemical parameters from commercially available Sangiovese wines, by performing a soft independent modeling of class analogy (SIMCA).

Keywords: anthocyanin profile, clone plasticity, ripening, Sangiovese, SIMCA, wine quality

1. INTRODUCTION

Sangiovese is the most widespread Italian red cultivar and, according to the last agricultural census of the Ministry of Agricultural, Food, and Forestry Policies (http://catalogoviti.politicheagricole.it), the total area planted with Sangiovese was 69787 ha, equivalent to 10.3% of the total area of vineyards in Italy. 47% of these vineyards are in Tuscany, producing 92.5% of the Sangiovese world output. Sangiovese constitutes the basis of internationally known wines such as Chianti, Brunello di Montalcino, Nobile di Montepulciano, and, furthermore, its use is allowed in the production of 11 DOCG (Denominazione di Origine Controllata e Garantita), 103 DOC (Denominazione di Origine Controllata) and 99 IGT (Indicazione Geografica Tipica) wines all over Italy.
The selective pressure carried out by mankind over the centuries in different growing conditions has induced the diversification of Sangiovese into many clones (CAMPOSTRINI *et al*., 1995).
Nowadays, 116 clones of Sangiovese are listed in the Italian National Registry of Grapevine Varieties. Clone performances vary with environmental conditions according to the interaction between the grapevine, weather and soil (BARBEAU *et al*., 1999) and they can influence wine quality and typicality. Some authors (TONIETTO and CARBONNEAU, 2004) consider that climate is the most dominant factor in determining grape quality and that is responsible for the *terroir* effect.
A few studies have been conducted on the physiological and productive response of Sangiovese clones to environmental and pedoclimatic factors. On the other hand, while some authors have focused on the relationship between the Sangiovese grape composition and different grapevine growth conditions, they only monitored standard parameters such as pH, titratable acidity, and sugar content (DI COLLALTO *et al*., 2000).
However, some studies on other cultivars, such as Tempranillo, Pinot Noir, Merlot, and Cabernet Sauvignon, have shown that, within the same grape variety, different clones can be distinguished by comparing their field performances including yield components (number and weight of clusters and berries, pruning weight) and the chemical compositions of grapes including anthocyanin content (REVILLA *et al*., 2009, FIDELIBUS *et al*., 2006, FIDELIBUS *et al*., 2007, CASTAGNOLI *et al*., 2006, AROZARENA *et al*., 2002, RANKOVIĆ-VASIĆ *et al*., 2015) and anthocyanin profiles (GUIDONI *et al*. 2002, RYAN and REVILLA, 2003, DOWNEY *et al*., 2006, GONZÁLEZ-NEVES *et al*., 2004, ORTEGA-REGULES *et al*., 2006).
These studies demonstrated that climate and soil are crucial factors in determining the final composition of grapes. However, it is not easy to predict how these changes could affect the final characteristics of wines. Integrated approaches can identify the relationship between grape chemical features, wine chemical composition, and wine sensory attributes, in order to predict wine flavor from grape composition and provide a practical tool for guiding the winemaking process (ZANONI *et al*., 2010, FORDE *et al*., 2011).
Many studies (CADOT *et al*., 2012, KONTOUDAKIS *et al*., 2011, KOUNDOURAS *et al*., 2006, RISTIC *et al*., 2010) have proposed different methodological approaches to measure the qualitative characteristics of grapes and the related wines, but a complete methodological approach to assess the eligibility of grapes in order to obtain a wine with well-defined characteristics has not been taken into account by these authors. A recent study proposed a multivariate statistical approach to relate grape features, and enological and agronomical practices with the composition of wines, in order to provide a practical tool to manage the vineyard and the winemaking process and preserve or enhance wine typicality (CANUTI *et al*., 2017).
The aim of this study was to create a tool capable of discriminating grapes, depending on their chemical composition, according to a given enological objective. For this purpose, a

grape eligibility model (GEM) was developed by computing the chemical parameters of commercially available Sangiovese wines, grapes, and the relevant wines from previous experiments by performing a soft independent modeling of class analogy (SIMCA). The model was then applied to assess the enological eligibility of seven Sangiovese clones grown in four vineyards in different growing areas of the Chianti Classico region in Tuscany.

2. MATERIALS AND METHODS

2.1. Grape samples

The present study was carried out using seven *Vitis vinifera* cv. Sangiovese clones listed in the Italian National Registry of Grapevine Varieties as reported in Table 1.

Table 1. Sangiovese clones studied for performance evaluation.

Registered clones	Code	Area of origin
SS-F9-A5-48	F9	Lamole (Florence - Tuscany, Italy)
Rauscedo 24	R24	Predappio (Forlì Cesena - Emilia Romagna, Italy)
Montalcino 42	M42	Montalcino (Siena - Tuscany, Italy)
AP-SG-1	AP1	Cossignano (Ascoli Piceno - Marche, Italy)
Peccioli 1	PEC	Peccioli (Pisa - Tuscany, Italy)
Rauscedo 10	R10	Lamole (Florence - Tuscany, Italy)
SG-12T	12T	Predappio (Forlì Cesena - Emilia Romagna, Italy)

The vineyards were located in four different estates in the Chianti Classico DOCG area in Tuscany. These were situated in the provinces of Florence (Panzano and Greve in Chianti) and Siena (Castellina in Chianti and Castelnuovo in Berardenga) and coded with the letters A, B, C, and D respectively. Each vineyard was planted in 1990 and has an average surface area of 1.7 hectares. The vines were spaced 2.8 m (between rows) × 1.0 m (between vines in the row), resulting in a density of 3571 vines/ha, in a randomized block design with a minimum of three replicates. Each clone was grafted on to 420 A (*Vitis berlandieri* x *Vitis riparia*) rootstock. The vines, with a permanent unilateral cordon, were spur-pruned and trained to a vertical shoot-position. The same soil management techniques were applied in all the vineyards, in which the rows were alternately covered with a spontaneous permanent grass and tilled. No irrigation system was installed.
The meteorological indices, based on the data recorded by the nearest climate stations to the experimental vineyards and the characteristics of the soils, are available at the reader's request.
Grapes were harvested for sampling at three different ripening stages during the 2005 vintage (September 10th, 20th and 30th). In 2005, rain events were quite intense at the beginning of September, as indicated by the data collected at the local meteorological stations. On each sampling date, 2 kg-samples of grapes were collected from portions of different clusters picked randomly from all the randomized blocks and analyzed.

2.2. Chemicals

The acetonitrile and *O*-phosphoric acid (HPLC grade) were purchased from Panreac (Barcelona, Spain). The malvidin-3-monoglucoside (M3MG) (HPLC grade) was purchased from Extrasynthèse (Genay, France). All of the other chemicals were of the highest purity available and were purchased from Sigma-Aldrich (Milan, Italy).

2.3. Instrumentation

The HPLC analyses were carried out on a Perkin-Elmer 200 LC Series equipped with an autosampler and a diode-array detector (Perkin Elmer, Shelton, CT, USA). The ultraviolet-visible (UV/vis) absorbance of the samples was measured on a Perkin Elmer Lambda 35 UV/Vis spectrophotometer (Perkin Elmer, Shelton, CT, USA).

2.4. Grape analysis

The technological ripeness of the grapes was measured following official OIV methods (Compendium of International Methods of Analysis – OIV – Oeno 21/2004). Two hundred berries were pressed to extract their juice. The juice sugar contents (Brix), titratable acidity (g/L), and pH were measured after centrifugation of the juice at 3000 rpm for 10 min. The berry weight was determined as the ratio between the total weight and the number of berries. Phenolic maturity was measured as described by SAINT-CRIQ *et al*. (1998).
The anthocyanin profiles (expressed as relative abundance of the different anthocyanins) of the same grape extracts at pH 3.2 were determined by HPLC, according to a previously published method (PENG *et al*., 2002) used to determine the phenolic maturity by acquiring a chromatogram at 520 nm. At the same time, the tannin contents (expressed as peak height) were determined by acquiring a chromatogram at 280 nm, as described in CANUTI *et al*. (2012). Chromatograms were acquired, recorded, and processed using Total Chrome Navigator software (Perkin Elmer).

2.5. Analysis of commercial and experimental wines

Standard parameters were measured in the wines following official EU methods (Official Methods of Wine Analysis, Reg. 440/2003). Color intensity (CI) and hue (Hue) were measured according to GLORIES (1984) and the total phenols index (TPI) was measured as described by RIBEREAU-GAYON (1970).
Monomer anthocyanin contents, expressed as mg/L of malvidin-3-monoglucoside (M3MG), colored polymeric pigments (CPP) expressed as mg/L of M3MG, and tannins (expressed as peak height) were determined by HPLC (PENG *et al*., 2002). Chromatograms were acquired at 520 and 280 nm respectively, using the same HPLC parameters reported in the grape analysis section.

2.6. Statistical analysis

The analysis of variance (ANOVA) was performed using Statgraphics Centurion (Ver. XV, StatPoint Technologies, Warrenton, VA), considering clones, growing area, and sampling date as factors. Principal Component Analysis (PCA) and the Soft Independent Modelling of Class Analogy (SIMCA) were performed using Unscrambler (V10.3, CAMO Process AS, Oslo, Norway).
The SIMCA analysis enables the assessment of which factors are decisive in determining the classification. Indeed, in this classification method, each class is described by an

independent principal component analysis model. New samples are classified on the basis of their fit with the different PCA models. The optimal number of PCs for each model is chosen independently since the classes may exhibit different shapes and structures. For new samples the residuals and scores are calculated for each PCA model. The residuals provide information on the ability of each model to describe the new data, like a sort of object-to-model distance, while the scores can be combined to measure the distance between the object and the model center.

2.7. Grape quality model and evaluation of clone performance

A model evaluating the grape quality, and the consequent performance of the clones, was built in order to provide an objective tool to assess the grapes' eligibility for winemaking. The classification method (SIMCA) consisted of describing each class of samples (wines and grapes), identified by their chemical composition, in independent Principal Component Analysis (PCA) models. Grape and wine samples were classified on the basis of their membership limit within the different PCA models (WOLD and SJOSTROM, 1977). In particular, the methodology to build the model and evaluate the clone performances consisted of three phases as follows:

Phase 1 – Sangiovese wine eligibility model (WEM)

In Phase 1, a Sangiovese wine eligibility model was built which described the Chianti Classico region wines. The aim of this phase was to establish a definition of Sangiovese wines from the region. For this purpose, 37 commercial Chianti Classico DOCG wines (coded with numbers from 101 to 137) were analyzed to determine their alcohol contents, titratable acidity, pH, total phenol index, total anthocyanins, tannins, color intensity, and hue. Later, a global PCA was run considering all the parameters for the 37 commercial wines. All the wines used to build the WEM were produced with 100% Sangiovese grapes without oak contact and analyzed one year after the harvest.

Phase 2 – Sangiovese grape eligibility model (GEM)

The aim of Phase 2 was to create a grape eligibility model (GEM) starting from the WEM built in Phase 1. For this purpose, 30 Sangiovese grape samples from the Chianti Classico region were analyzed to determine their sugar content, pH, titratable acidity, total and extractable anthocyanins, cellular maturity index, phenolic richness, and tannins. The grapes were then vinified, on an industrial scale, to obtain 30 experimental Sangiovese wines (coded with numbers from 1 to 30) that were analyzed, after aging for one year, to determine their alcohol contents, titratable acidity, pH, total phenol index, total anthocyanins, tannins, color intensity, and hue.
Finally, the chemical composition parameters of 30 Sangiovese grape samples and the related experimental wines were statistically analyzed in order to correlate the grapes to wine composition. A global PCA of the experimental wines was performed and the resulting model was compared with a SIMCA analysis to assess which wines fitted the WEM.
The grape samples whose wines fitted the WEM obtained in *Phase 1* were used to create the Grape Eligibility Model (GEM) by running a global PCA which considered all of the grape parameters.

Phase 3 – Evaluation of clone performance

In order to evaluate the clones' performances, the grape samples were classified according to their composition using a SIMCA analysis to assess which grapes fitted the GEM.
Outliers were detected during the exploratory analysis by calculating the Hotelling's T^2 distance diagnostic tool for each class of samples.

3. RESULTS

3.1. Grape clone characteristics

The data collected from grape sample analyses at three different ripening stages were statistically analyzed and the results are reported in Table 2.

Table 2. F-values and interactions for chemical parameters analyzed in the grape samples.

Variable	SD	C	GA	C x GA	GA x SD	C x SD
Sugar	13.97***	2.86*	70.43***	4.46***	4.25***	3.79***
pH	15.45***	5.65***	21.08***	1.23 ns	1.48 ns	1.53 ns
Titratable acidity	65.61***	14.35***	112.52***	6.43***	4.11***	9.24***
Berry weight	4.02*	13.78***	93.69***	11.52***	5.76***	5.01***
Total potential in anthocyanins	14.37***	23.41***	54.05***	13.06***	13.57***	5.00***
Extractable anthocyanins	10.46***	20.47***	70.42***	10.50***	9.89***	5.21***
Cellular maturity index	22.66***	6.09***	18.27***	7.10***	6.65***	6.26***
Phenolic richness	6.48**	5.15***	7.67***	2.87***	2.58*	2.08*
Di/Tri substituted anthocyanins ratio	21.82***	15.93***	92.03***	27.48***	7.90***	2.07*
Tannins	5.05*	46.30***	186.47***	46.18***	4.48***	5.39***

*P ≤ 0.05; **P ≤ 0.005; ***P ≤ 0.001; ns, not significant; C, clone; GA, growing area; SD, sampling date.

All the variables measured have been significantly affected by the factors clone (C), growing area (GA), sampling date (SD) factors and their interactions, with the exception of pH (Table 2). Furthermore, the ANOVA results indicate that GA was the most influential factor; in fact, the F-values of GA were the highest for all the considered grape parameters.
The C factor was found to be a determining factor for the concentrations of phenolic compounds (tannins, potential and total anthocyanins). As expected, the SD factor did not only influence the cellular maturity index but also the quality of the anthocyanins, modifying the di-substituted and tri-substituted pigment ratios.
With regard to the interactions between the various factors, the results suggest that the interaction between clone and growing area (C x GA) was the most significant, in relation to tannin contents (F = 46.18) and the Di/Tri index (F = 27.48).
The mean plots for the standard and phenolic parameters of the grape samples are shown in Figs. 1 and 2, respectively.
The berry weight (BW) provides a qualitative and quantitative indication about the berry size and the skin to pulp ratio. The most important factor that influenced BW (Table 2) was GA, which showed the highest F - value (93.69). According to Fig. 1, BW remained fairly constant during the ripening stage, indicating that the SD factor had less influence on BW (F – value = 4.02) if compared to the GA and C factors which had a higher statistically

significant influence (Table 2). In particular, clone F09 showed a higher BW in comparison to clone 12T, which produced smaller berries. Growing areas A and D seemed to stimulate the growth of the berry more than growing area B. Clone R24 had the highest berry weight variability, producing the lightest berries (1.24 g) in area B and the heaviest (2.37 g) in D. The clone PEC stood out in area C with an average weight of 2.35 g. Clones F09, AP1, and R10 produced grapes with a more constant berry weight in all the growing areas.

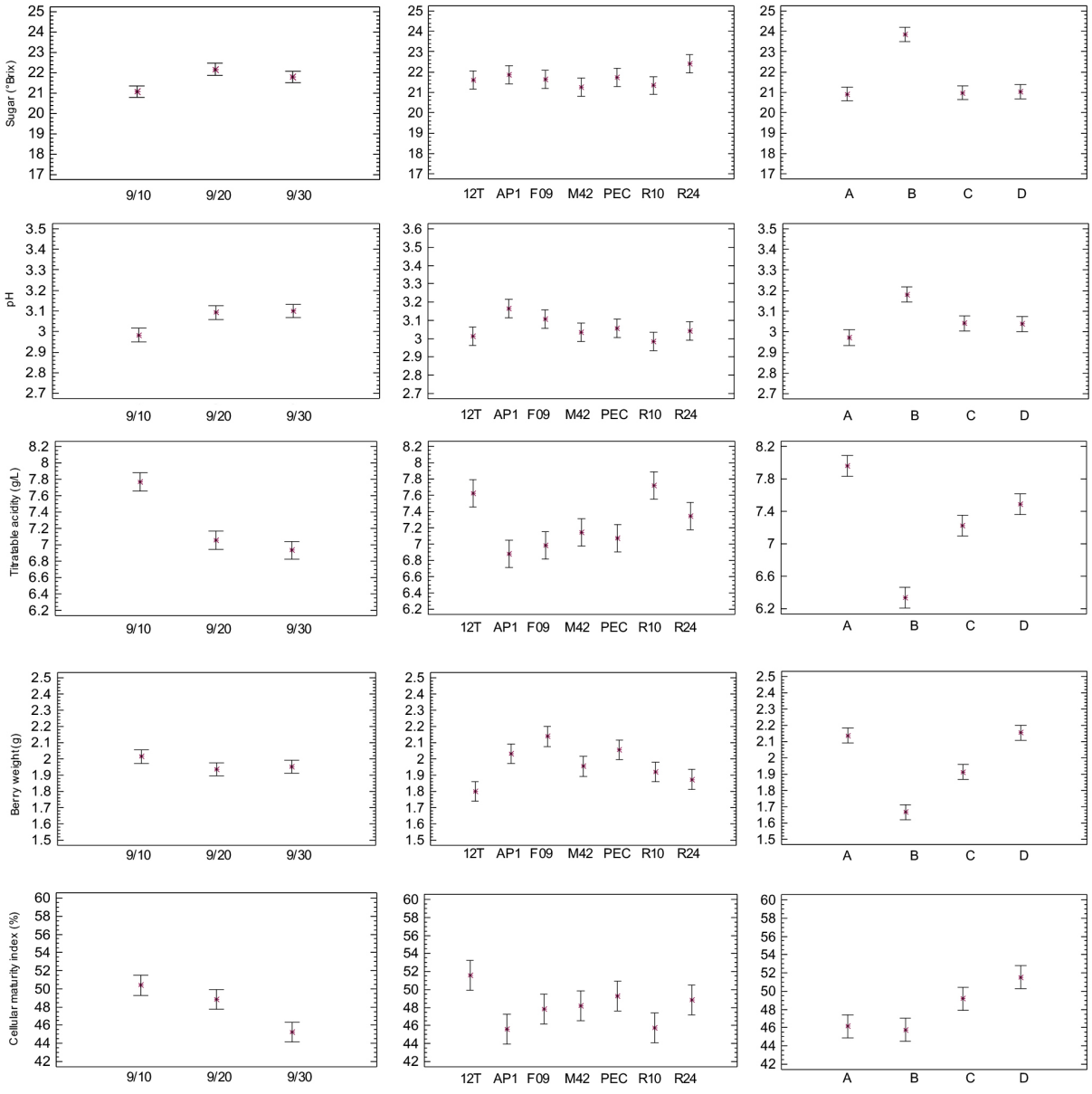

Figure 1. Mean plots of standard parameters of grape samples (sugar, pH, titratable acidity, berry weight, and cellular maturity index); significance at 95% confidence level according to Fisher's least significant difference (LSD) procedure. Bars represent LSD; total number of grape samples for each plot = 252.

All of the factors had a statistically significant influence in relation to the grapes' sugar content, and GA was the one with the greatest effect (F-value = 70.43). There were also significant effects when considering the interactions between factors. For example, Fig. 1

shows that the sugar content increased, as expected, during ripening, with clone R24 having the highest sugar content, and growing area B promoting the accumulation of sugars. The sugar content of the grapes produced by clones 12T and AP1 showed the most unstable and extreme values, which varied between 25.0 Brix in B and 20.5 in the other zones. R10 showed constant concentrations (21.3 Brix) in all the growing areas.

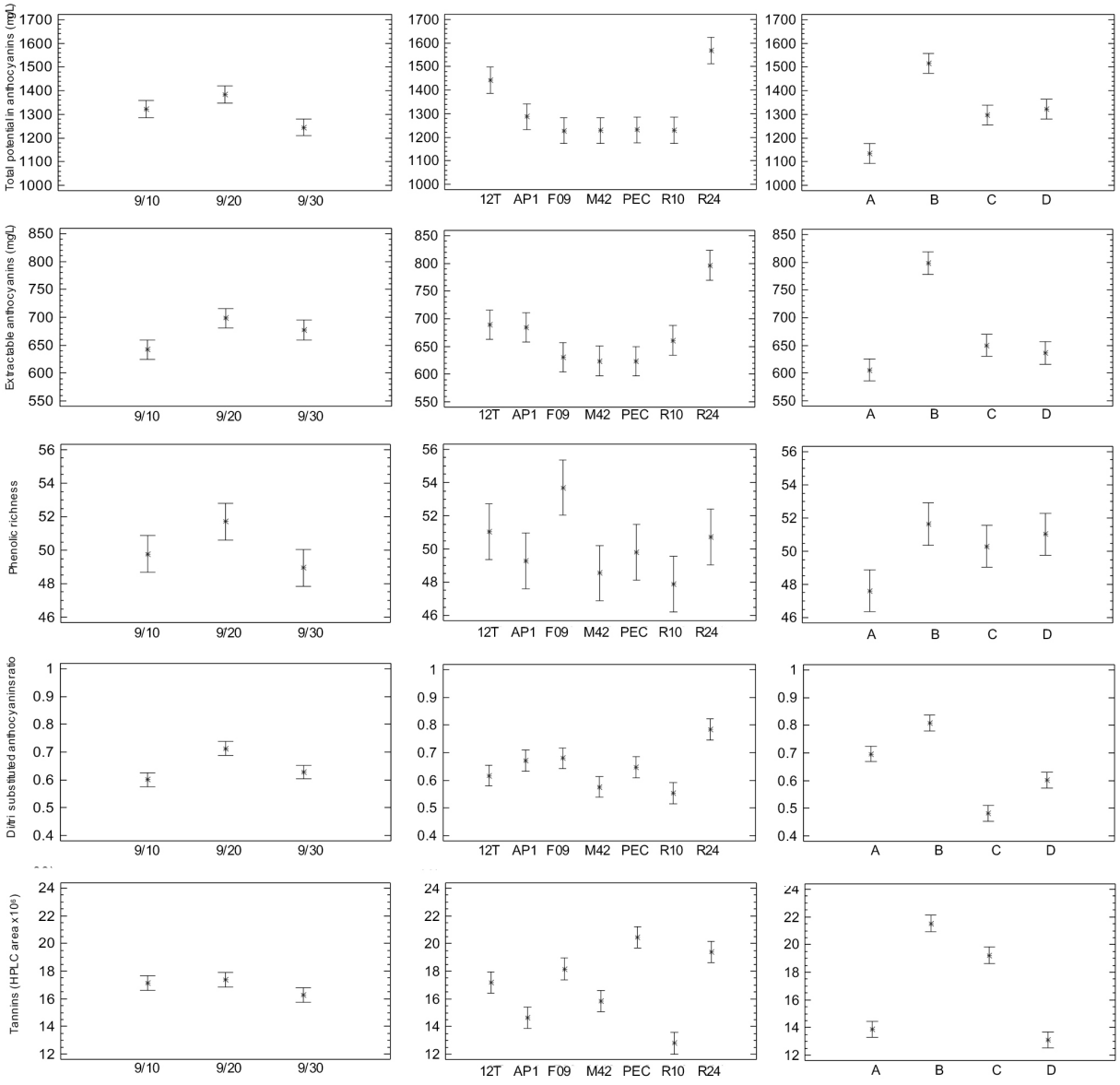

Figure 2. Mean plots of phenolic parameters of grape samples (total potential in anthocyanins, extractable anthocyanins, phenolic richness, di/tri substituted anthocyanins ratio, and tannins); significance at 95% confidence level according to Fisher's least significant difference (LSD) procedure. Bars represent LSD; total number of grape samples for each plot = 252.

Concerning the titratable acidity and pH (Table 2), the most determining factor was again GA (F - value = 112.52 and 21.58 respectively). Moreover, the titratable acidity decreased as the pH increased at the three different sampling dates (F = 65.61). Clone AP1 showed the highest pH and clones 12T and R10 the highest titratable acidity (Fig. 1).

Growing area B produced grapes with the highest pH and the lowest titratable acidity in comparison to the other GAs. The grapes grown in area A showed the highest titratable acidity and the lowest pH. The titratable acidity was similar for all the clones in growing area B, while the results showed a high variability in the other zones. F09 reached the lowest value of titratable acidity (6.65 g/L) in area D. Clone R10 displayed the highest values in zones A (8.59 g/L) and C (8.08 g/L). Moreover, in growing area A, clones 12T (8.72 g/L) and R24 (8.57 g/L) stood out for their high titratable acidity.

The cellular maturity index (EA%) measures the berries' ability to release anthocyanins: the lower the EA index value, the higher the extractable potential of the anthocyanins (SAINT CRIQ *et al*., 1998). The evolution of this factor at the different SDs (Fig. 1) highlighted a progressive cellular maturity that should result in a better release of the phenolic compounds during winemaking. Statistical analysis (Table 2) pointed out that EA% mostly depended on the SD (F = 22.66) and GA factors (F = 18.27) and that growing areas A and B promoted the ripening of the grape skin cells the most. Moreover, clone 12T showed the highest EA% (Fig. 1). The cellular maturity index was stable for clone PEC (49) while it was inconstant for F09 (38 in B and 51 in C) and M42 (40 in A and 56 in C). The EA% was the highest for all the clones in growing area D.

During the ripening of the grapes, there was a constant and significant increase in phenolic richness and total potential in anthocyanins from the first to the second SD and a significant decrease at the third SD (Fig. 2). Instead, the extractable anthocyanins increased over time, reaching the maximum at the second sampling date and remaining constant between the second and third SDs.

The tannin contents remained constant throughout the ripening stages; this result could indicate that the increase in the total amount of phenolic compounds was due to the accumulation of monomeric phenols (Fig. 2). However, clones PEC and R24 showed the highest tannin content, and there were significant differences induced by the GA factor, with growing areas A and D having the lowest values and areas B and C showing the highest value for tannin contents.

The largest differences in phenolic composition between the grapes emerged when considering GA as the main factor. In fact, the content of total potential (F = 54.05), extractable anthocyanins (F = 70.42) and tannins (F = 186.47) was higher in zone B and lower in all the other growing areas. Moreover, the differences in the levels of phenolic richness were less remarkable but still significant for growing areas B, C, and D, which showed higher values than area A.

Upon examining the chemical profile of the different Sangiovese clones, it is clear that there were differences among them due to the content of total anthocyanins but not at the level of phenolic richness. Clone R24 showed a different behavior when compared to all the other clones, resulting in significantly higher contents of total potential and extractable anthocyanins and tannins.

Regarding the clone x growing area interactions, clone F09 always showed similar levels of phenolic richness (54), while R24, with values that ranged between 47 in A and 56 in D, was the clone most influenced by the GA factor. The tannin content was similar for all the clones in areas A and B; a higher variability was observed in the other zones where clones PEC, R24 (in B), and F09 (in C) stood out with higher values.

The anthocyanin content, both potential and extractable, showed the highest variability in zone B where clones 12T (1879 and 946 mg/kg) and R24 (2011 and 983 mg/kg) stood out with the highest values. In area B, the values were higher for all the clones with the exception of F09 (1043 and 642 mg/kg) which, on the contrary, was the richest in anthocyanins in growing area C (1609 and 744 mg/kg).

Different considerations should be made regarding the ratio between the di- and tri-substituted anthocyanins (Di/Tri) and the anthocyanin profiles of the different clones.

Clone R24 showed the highest Di/Tri value and growing areas A and B showed larger levels of Di/Tri in comparison to areas C and D (Fig. 2).
Regarding the relative abundance of anthocyanins, it was seen that the pigments were mainly represented by delphinidin-3-O-glucoside (DEL), cyanidin-3-O-glucoside (CYA), peonidin-3-O-glucoside (PEO), petunidin-3-O-glucoside (PET), and malvidin-3-O-glucoside (MAL). In agreement with results reported elsewhere (MATTIVI *et al*., 2006, ARAPITSAS *et al*., 2012), the acylated anthocyanins were found in very low amounts (sum of total relative area below 2% of the total anthocyanin content, data not shown).
Due to the low amounts of acylated pigments in the Sangiovese cultivar, only glucoside anthocyanins were taken into consideration for the statistical analysis (DEL, CYA, PEO, PET and MAL).
The variability in Di/Tri levels, according to the multifactor ANOVA, was affected by all the factors taken into consideration (Table 2). All the clones had similar Di/Tri values with the exception of clone R24 (F = 15.93), which showed a higher value (Fig. 2). Small variations occurred during the ripening period (F = 21.82). In this case too, the growing area was the most influential factor on this parameter (F = 92.03), with areas A and B showing the highest values of the index. The Di/Tri ratio was similar for all the clones in growing areas A and C while in area B the values varied between 1.37 for clone R24 and 0.49 for R10.
Upon analyzing the anthocyanin profile (Table 3), MAL resulted the major component of the anthocyanin pool present in the Sangiovese grapes from all the different clones; while DEL was the smallest component.

Table 3. Grape clone anthocyanin percentages and significance[1].

	Anthocyanins					
Factors	**Del**	**Cya**	**Pet**	**Peo**	**Mal**	**Σ tri-sub**
Clone						
12T	9.5^{c}	20.5^{a}	13.3^{cd}	16.9^{b}	39.7^{bc}	62.5^{ab}
AP1	9.1^{b}	19.5^{a}	12.8^{b}	19.8^{d}	38.7^{b}	60.6^{ab}
F09	6.8^{a}	19.9^{a}	10.4^{a}	18.6^{c}	44.1^{d}	61.3^{ab}
M42	9.7^{c}	19.5^{a}	13.4^{d}	16.5^{b}	41.0^{c}	64.1^{b}
PEC	9.4^{c}	23.7^{b}	13.7^{d}	14.8^{a}	38.1^{b}	61.2^{ab}
R10	10.2^{d}	19.9^{a}	14.1^{c}	14.3^{a}	41.2^{c}	65.5^{b}
R24	10.3^{d}	24.5^{a}	12.9^{bc}	17.4^{b}	34.6^{a}	57.8^{a}
Growing area						
A	9.9^{c}	24.2^{c}	13.7^{b}	16.2^{b}	35.8^{a}	59.4^{a}
B	8.9^{b}	24.7^{c}	12.3^{a}	18.7^{d}	35.2^{a}	56.4^{a}
C	9.9^{c}	16.6^{a}	13.8^{b}	15.2^{a}	44.4^{b}	68.1^{c}
D	8.5^{a}	18.9^{b}	12.0^{a}	17.5^{c}	43.0^{b}	63.5^{b}
Sampling date						
9/10	9.4^{a}	19.6^{a}	13.0^{a}	16.5^{a}	41.3^{c}	63.7^{b}
9/20	9.3^{a}	22.8^{a}	12.8^{a}	17.3^{b}	37.6^{a}	59.7^{a}
9/30	9.3^{a}	20.8^{a}	12.9^{a}	16.9^{ab}	39.9^{b}	62.1^{ab}

[1]Different letters within the same row mean significant differences (significance at 95% confidence level according to Fisher's least significant difference (LSD) procedure). DEL: delphinidin-3-O-glucoside; CYA: cyanidin-3-O-glucoside; PEO: peonidin-3-O-glucoside; PET: petunidin-3-O-glucoside; MAL: malvidin-3-O-glucoside; Σ tri-sub: sum of tri-substituted anthocyanins.

CYA was the second most abundant anthocyanin in Sangiovese grapes. The sum of the tri-substituted anthocyanins (DEL, PET and MAL) ranged between 57.8-65.5%. The results showed that the average anthocyanin profile of Sangiovese corresponded to the one described in the literature (MATTIVI *et al*., 2006, ARAPITSAS *et al*., 2012). However, significant differences in the anthocyanin composition of the grapes were related to C, GA, and SD (Table 3).

3.2. Grape quality model and evaluation of clone performance

Phase 1 – Sangiovese wine eligibility model (WEM)

The model was built by running a PCA which considered the chemical parameters of 37 commercial Sangiovese wines from the Chianti Classico region.
The scores of the wines relative to the two first PCs and the Hotelling T^2 Ellipse (95% confidence level) are represented in Fig. 3.

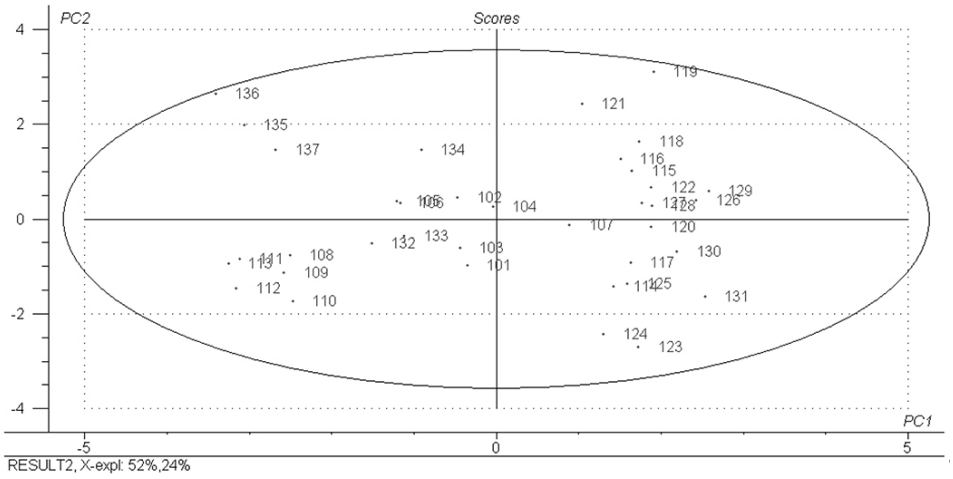

Figure 3. Principal Component Analysis (PCA) and Hotelling T^2 Ellipse of the commercial wines, numbered 101 to 137.

By encircling all the samples, the Hotelling statistic evidenced the absence of outliers among the wines. The statistical analysis confirmed, moreover, a homogeneity between the samples, stating that they reasonably belong to the same class. For the construction of the model, the number of PCs was chosen to reach a level of explained variance between 80 and 90%. For the WEM, three principal components that accounted for 84.11% of the total variance were considered adequate.

Phase 2 – Sangiovese grape eligibility model (GEM)

The WEM built in Phase 1 was used to classify 30 experimental wines according to their analytical profiles. The SIMCA analysis allowed the identification of 23 wines that fitted the model criteria, while rejecting wine samples 6, 13, 20, 22, 23, 27, and 28 (Table 4).
The grapes used in the experimental wines that resulted eligible by applying the WEM were considered suitable for the construction of a grape eligibility model (GEM). The

analytical parameters of the 23 grape samples were then used to calculate a PCA in order to set up the GEM.

Table 4. Classification of the experimental wines (SIMCA). Numbers 1 to 30 represent the wine samples. (●) wine which fitted the WEM, (-) wine which did not fit the WEM.

Experimental wine		Experimental wine	
1	●	16	●
2	●	17	●
3	●	18	●
4	●	19	●
5	●	20	-
6	-	21	●
7	●	22	-
8	●	23	-
9	●	24	●
10	●	25	●
11	●	26	●
12	●	27	-
13	-	28	-
14	●	29	●
15	●	30	●

Fig. 4 reports the PCA performed with the chemical parameters of the eligible grapes previously selected using the SIMCA analysis. In this case too, no outliers were found according to the Hotelling T^2 Test, confirming the homogeneity among the samples and indicating that they reasonably belong to the same class.

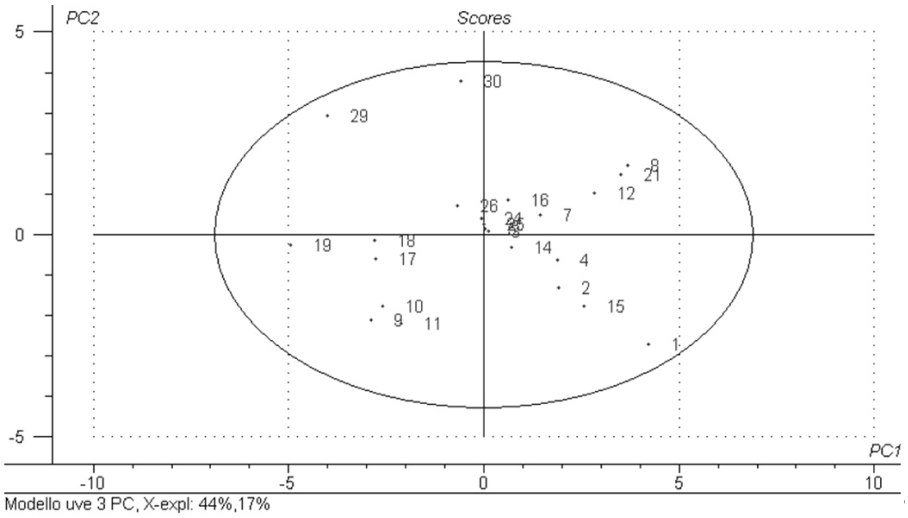

Figure 4. Principal Component Analysis (PCA) and Hotelling T^2 Ellipse of the eligible grapes. The numbers inside the ellipse represent the grape samples whose wines fitted the WEM.

Similarly, as was done for the construction of the WEM, to build the GEM the number of PCs was chosen to reach a level of explained variance between 80 and 90%. For the purpose of this study, four PCs, which accounted for 81.72% of the total variance, were considered adequate.
The SIMCA analysis also allowed us to assess which factors are decisive in determining the classification of wines. The modeling power represents the contribution of each factor to building the model expressed as the variance of each variable. Any variable having a modeling power higher than 0.3 was considered relevant in the model. In our study all the considered variables were relevant and in particular, hue (0.709), color intensity (0.704), monomer anthocyanins (0.699), and titratable acidity (0.628) were the variables with the greatest modeling power for the wines, followed by total phenol index (0.584), alcohol content (0.554), tannins (0.504), and pH (0.498).

Phase 3 – Evaluation of clone performance

Lastly, the chemical profile of the grape clones was compared with the GEM to classify their performance as a function of the clone, the sampling date, and the different growing areas. The results indicating which grapes had the chemical characteristics to fit the GEM are reported in Table 5.

Table 5. Classification of the grapes (SIMCA) as a function of the clone (12T, AP1, F09, M42, PEC, R10, R24), the sampling date (9/10, 9/20, 9/30) and the different growing area (A, B, C, D); (●) grapes fitting the GEM, (-) grapes not fitting the GEM.

	A			B			C			D		
	9/10	9/20	9/30	9/10	9/20	9/30	9/10	9/20	9/30	9/10	9/20	9/30
12T	-	-	●	-	-	-	-	●	●	-	-	-
AP1	-	-	-	●	-	-	●	●	-	●	●	-
F09	-	●	●	●	-	-	●	-	-	-	-	●
M42	-	-	-	●	●	-	-	-	●	-	-	-
PEC	-	●	-	-	-	-	-	●	-	-	●	●
R10	-	-	-	●	●	●	-	-	-	-	-	●
R24	-	-	-	●	-	●	-	-	●	-	●	-

The clones showed a different performance according to the different growing areas and their ripening stage. Considering the growing area as a factor, it can be seen that in zone A the ideal ripeness was reached in only four cases. In zone A, furthermore, only three clones were in accord with the parameters defined by the model: 12T, F09, and PEC. Area C, on the contrary, showed the highest number of clones that produced grapes with the desired characteristics.
With regard to the performances of the individual clones, F09 resulted the only clone able to fit the model in all the growing areas on at least one of the sampling dates.
The grapes from clones 12T, M42 and R10 fitted the model in two distinct zones: clone 12T in areas A and C; clone M42 in areas B and C; and clone R10 in areas B and D; the other clones (AP1 and R24) reached the maturity required by the model in three growing areas (areas B, C and D for clones AP1 and R24, and areas A, C, and D for clone PEC).
Total potential in anthocyanins (0.797), extractable anthocyanins (0.699), and tannins (0.684) were the variables with the greatest modeling power for the grapes (expressed as

the variance of each variable) followed by cellular maturity index EA% (0.674), phenolic richness (0.607), titratable acidity (0.562), pH (0.505), and sugar (0.497).
These results show the strong influence of the soil and climate on the ripeness parameters of Sangiovese grapes. In growing area B five clones already fitted the model in the first sampling period, producing the fastest ripening. In this case, the grapes tended to exit the eligibility model over time. This highlights how the GEM determined the unsuitability of grapes due to over-ripening, and therefore identified precise periods within which the quality of the grapes reaches its peak in relation to a particular enological target (WEM).
Table 5 also shows how the model determined not only thresholds of acceptability but a real eligibility criterion. Indeed, when all these variables are taken into account together, throughout the model, it creates a "space of acceptability where grapes transit over time," with some of the grapes entering the space as they ripen while others, which were initially acceptable, are later expelled from the space of suitability by the model, because of over-ripening.
It should be emphasized that the study was carried out in a single year, in order to propose a methodological approach. On the other hand, an adequate number of replicates over the years, taking into account the seasonal trends, should be analyzed when studying the performance of clones in a given area.

4. CONCLUSIONS

In the present study, grapes from seven Sangiovese grape clones cultivated in the Chianti Classico region in Tuscany were chemically characterized and compared, over the ripening period, to understand if non-genetic factors could affect the performances of different clones.
In the first part of the study, the results showed that grape characteristics were influenced by all the factors considered: clone, growing area, and sampling date. The largest differences between the grapes, according to the phenolic composition, emerged when considering the growing area as a factor and the total anthocyanin content as a variable. The anthocyanin profiles were also affected by the different growing conditions and clones; the most abundant anthocyanins were malvidin-3-O-glucoside and cyanidin-3-O-glucoside while acylated anthocyanins were detected in a very low amount (less than 2%), confirming the results for Sangiovese wines reported elsewhere.
In the second part of the study, a statistical model was developed to evaluate the impact of Sangiovese variability on grape eligibility referring to a given enological target.
For this purpose, a Chianti Classico Sangiovese wine reference model (WEM) was developed with the chemical characteristics of commercial wines from the same area. By comparing the composition of the experimental wines that fitted the WEM with the relevant grapes, a model (GEM) was defined that allowed us to discriminate the Sangiovese grapes on the basis of their suitability to produce wines with the desired properties. The model could be expanded by inserting additional features such as aroma compounds or by using quick analysis methods such as FT-NIR that can easily predict some chemical grape and wine parameters.
With an adequate number of replicates, the proposed approach could be useful in zoning studies or in determining the performance of different varieties or clones with the goal of producing a typical Sangiovese wine of the Chianti Classico region. Moreover, it could be implemented as a more rational use of available analytical data both to monitor grape maturation trends and to improve the management of winemaking processes by transforming chemical analysis databases into active decision-making tools.

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REFERENCES

Arapitsas P., Perenzoni D., Nicolini G. and Mattivi F. 2012. Study of Sangiovese wines pigment profile by UHPLC-MS/MS. J. Agric. Food Chem. 60:10461-10471.

Arozarena I., Ayestarán B., Cantalejo M.J., Navarro M., Vera M., Abril I. and Casp A. 2002. Anthocyanin composition of Tempranillo, Garnacha and Cabernet Sauvignon grapes from high- and low-quality vineyards over two years. Eur. Food Res. Tech. 214:303-309.

Barbeau G., Cousin M., Blin A., Panneau J-P., Bouvet M-H. and Mège A. 1999. Méthodologie de sélection clonale chez la vigne (*Vitis vinifera*). Priese en compte de la précocité du cycle et de l'interaction clone x terroir. Bull. OIV. 72:731-751.

Cadot Y., Caillé S., Samson A., Barbeau G. and Cheynier V. 2012: Sensory representation of typicality of Cabernet franc wines related to phenolic composition: Impact of ripening stage and maceration time. Anal. Chim. Acta 732:91-99.

Campostrini F., De Micheli L., Bogoni M. and Scienza A. 1995. Study of genetic variability of Sangiovese ecotypes as a tool for new strategies in clonal selection. In: Proceedings of the International Symposium of Clonal Selection. J.A. Wolpert, M.A. Walker, D. Roberts (Eds), pp. 105-110. Portland, Oregon.

Canuti V., Puccioni S., Giovani G., Salmi M., Rosi I. and Bertuccioli M. 2012. Effect of oenotannin addition on the composition of Sangiovese wines from grapes with different characteristics. Am. J. Enol. Vitic. 63:220-231.

Canuti V., Picchi M., Zanoni B., Fia G. and Bertuccioli M. 2017. A multivariate methodological approach to relate wine to characteristics of grape composition: the case of typicality. Am. J. Enol. Vitic.68:49-59.

Castagnoli S.P. and Vasconcelos,C. 2006. Field performance of 20 'Pinot noir' clones in the Willamette Valley of Oregon. Hort Technology 16:153-161.

Di Collalto G., Scalabrelli G. and Ferroni G. 2000. Primi risultati del comportamento bio-agronomico dei sei cloni di "Sangiovese" nella zona di produzione del "Morellino di Scansano". In: Proceedings of the Simposio Internazionale "Il Sangiovese", Palazzo dei Congressi, 15-17 febbraio 2000. pp. 265-270. Firenze, Italy.

Downey M.O., Dokoozlian N.K. and Krstic M.P., 2006. Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: A review of recent research. Am. J. Enol. Vitic. 57:257-268.

Fidelibus M.W., Christensen P.L., Katayama D.G. and Verdenal P-T., 2006. Yield components and fruit composition of six 'Cabernet Sauvignon' grapevine selections in the central San Joaquin Valley, California. J. Amer. Pomological Society 60:32-36.

Fidelibus M.W., Christensen P.L., Katayama D.G. Verdenal P-T. and Cathline K. 2007. Fruit characteristics of six merlot grapevine selections in the central San Joaquin Valley, California. Am. J. Enol. Vitic. 58:259-261.

Forde C.G., Cox A. Williams E.R. and Boss P.K. 2011. Associations between the sensory attributes and volatile composition of Cabernet Sauvignon wines and the volatile composition of the grapes used for their production. J. Agric. Food Chem. 59:2573-2583.

Glories Y. 1984. La couler des vins rouges. II Partie - mesure, origine et interpretation. Connaiss. La Vigne du Vin 18:253-271.

González-Neves G., Barreiro L., Gil G., Franco J., Ferrer M., Moutounet M. and Carbonneau A. 2004. Anthocyanic composition of Tannat grapes from the south region of Uruguay. Anal. Chim. Acta, 513:197-202.

Guidoni S., Allara P. and Schubert A. 2002. Effect of Cluster Thinning on Berry Skin Anthocyanin Composition of *Vitis vinifera* cv. Nebbiolo. Am. J. Enol. Vitic. 53:224-226.

Kontoudakis N., Esteruelas M., Fort F., Canals J.M., De Freitas V. and Zamora F. 2011. Influence of the heterogeneity of grape phenolic maturity on wine composition and quality. Food Chem. 124:767-774.

Koundouras S., Marinos V., Gkoulioti A., Kotseridis Y. and Van Leeuwen C. 2006. Influence of vineyard location and vine water status on fruit maturation of nonirrigated cv. Agiorgitiko (*Vitis vinifera* L.). Effects on wine phenolic and aroma components. J. Agric. Food Chem. 54:5077-5086.

Mattivi F., Guzzon R., Vrhovsek U., Stefanini M. and Velasco R. 2006. Metabolite profiling of grape: flavonols and anthocyanins. J. Agric. Food Chem. 54:7692-7702.

Official Methods of Wine Analysis, Reg. 440/2003.

Ortega-Regules A., Romero-Cascales I., Lòpez-Roca J.M., Ros-Garcìa J.M. and Gòmez-Plaza E. 2006. Anthocyanin fingerprint of grapes: environmental and genetic variations. J. Sci. Food Agric. 86:1460-1467.

Peng Z., Iland P.G., Oberholster A., Sefton M.A. and Waters E.J. 2002. Analysis of pigmented polymers in red wine by reverse phase HPLC. Aust. J. Grape Wine Res. 8:70-75.

Ranković-Vasić Z., Nikolić D., Atanacković Z., Sivčev B. and Ruml M. 2015. Characterization and adaptation of some 'Pinot Noir' clones to the environmental conditions of Serbian grape growing regions. Vitis 54:147-149.

Revilla E., García-Beneytez E., Cabello, F. 2009. Anthocyanin fingerprint of clones of Tempranillo grapes and wines made with them. Aust. J. Grape Wine Res. 15:70-78.

Ribereau-Gayon P. 1970. Le dosage des composés phénoliques totaux dans les vins rouges. Chim. Anal. 52:627-631.

Ristic R., Bindon K., Francis L.I., Herderic M.J. and Iland P.G. 2010. Flavonoids and C13-norisoprenoids in *Vitis vinifera* L. cv. Shiraz: relationships between grape and wine composition, wine colour and wine sensory properties. Aust. J. Grape Wine Res. 16:369-388.

Ryan J.M. and Revilla E. 2003. Anthocyanin composition of Cabernet Sauvignon and Tempranillo grapes at different stages of ripening. J. Agric. Food Chem. 51:3372-3378.

Saint-Criq de Gaulejac N., Vivas N. and Glories Y. 1998. Maturité phénolique: definition et control. Rev. Française d'Œnologie 173:14-21.

Tonietto J. and Carbonneau A. 2004. A multicriteria climatic classification system for grape-growing regions worldwide. Agric. Forest Meteorol. 124:81-97.

Wold S. and Sjostrom M. 1977. SIMCA: a method for analyzing chemical data in terms of similarity and analogy. Chemometr Theory Appl 52:243-282.

Zanoni B., Siliani S., Canuti V., Rosi I. and Bertuccioli M. 2010. A kinetic study on extraction and transformation phenomena of phenolic compounds during red wine fermentation. Int. J. Food Sci. & Tech. 45(10):2080-2088.

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