OENOLOGICAL ASPECTS OF THREE BIOTYPES OF COLORINO

Sergio Puccioni, Franco Giannetti, Alessandra Zombardo, Anna Maria Epifani, Paolo Storchi

Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Unità di Ricerca per la Viticoltura, Viale Santa Margherita 80, 52100 Arezzo (Italy).

sergio.puccioni@entecra.it

Introduction

In the recent past, there has been a long, but steady, abandonment of many grape varieties with the risk of the disappearance of many native vines. However, the need to develop typical wines, i.e. wines attributable to an area of production or to a winery style, has renewed the interest on the rediscovery of these cultivars. Colorino is an ancient Tuscan grape variety traditionally used to improve the colour of Sangiovese wines. Although Colorino production is marginal, according to A.R.T.E.A. in 2008 in Tuscany 347 ha were cultivated with this grapevine (A.R.T.E.A., 2015), it is blended with Sangiovese wine in some of the most important Tuscan DOCG, such as Chianti, Chianti Classico and Nobile di Montepulciano. It is sometimes vinified alone to obtain deeply coloured and tannic wines (Robinson et al., 2013). In addition to Colorino there are other Tuscan native coloured grape varieties: Abrusco and Abrostine. Similarly, they are used to enhance Sangiovese wines colour. Abrusco and Abrostine are rarely cultivated and they are at risk of extinction, since less than 10 ha are dedicated to this grapevine all over Italy.

Colorino is included in the register of varieties and clones of vines of the Italian Ministry of Agricultural, Food and Forestry Policies (MiPAAF) since 1970 and, currently, seven different clones have been registered. Abrusco was added to the list in 1999 as a distinct variety. However, from the same database of the Ministry, it results that Colorino and Abrusco have identical genetic profiles (MiPAAF, 2015) and, for this reason, they can be considered biotypes of the same variety. On the other hand, some molecular and morphological studies confirmed the different nature of these grapevines. Regarding Abrostine, there is a general agreement to consider it, from a genetic and structural point of view, a clone or a synonym of Abrusco (Vignani et al., 2008; Mancuso et al., 1998). In this work some oenological aspects of three biotypes of Colorino (Colorino del Valdarno, Abrusco and Abrostine) preserved in the vineyard collection of CRA-VIC in Arezzo (Italy), are investigated. The chemical characteristics of the grapes of Colorino biotypes and their wines, produced both alone and in blends with Sangiovese, were taken into consideration. Besides the technological parameters of grapes and the relative wines, particular attention was paid to the study of the anthocyanins profiles of grape skins, in order to highlight possible differences between the biotypes, since these features have not been investigated in previous studies (Mattivi et al., 1990; Ortega et al., 2006).

Materials and methods

For three years (2011-2013), grape samples of Colorino del Valdarno, Abrostine and Abrusco were compared to Sangiovese, analyzing sugar contents, total acidity, polyphenols and anthocyanins concentrations. The samples were processed according to the official methods of the International Organization of Vine and Wine (O.I.V., 2015) and Di Stefano (1989). Anthocyanins profiles were determined on grape skin extracts according to the method described by Bucelli et al. (1995). During the three vintages, furthermore, microvinifications (5 kg of grape each) were conducted to assess the oenological characteristics of the Colorino biotypes.

In 2014, moreover, the grapes from Abrostine, Abrusco and Colorino del Valdarno were fermented mixed with Sangiovese grapes with a 50% ratio. These were compared with the varietal wines in order to determine the effects of the blending on the phenolic compositions during the alcoholic fermentation. All the vinifications followed the same protocol and were triplicated. The maceration lasted 10 days and the cap was punched down twice a day; sulphur dioxide was added in order to have a concentration of 70 mg/L after the malolactic fermentation.

As for the grapes, the standard analysis of the wines were performed according to the protocols proposed by O.I.V. and the phenolic compositions were determined as described by Di Stefano (1997) and Glories (1984).

Results

In Figures 1 and 2, the values related to the ripeness of the grapes samples are represented. In the considered vintages, no significant differences in sugar content (Brix) and pH were found. Sangiovese grapes showed total acidity values slightly lower than Colorino biotypes and, as expected, Colorino del Valdarno, Abrusco and Abrostine had higher phenolic compounds content than Sangiovese. Colorino del Valdarno can be distinguished from the other coloured biotypes for a major concentration of total polyphenols (Fig.3).

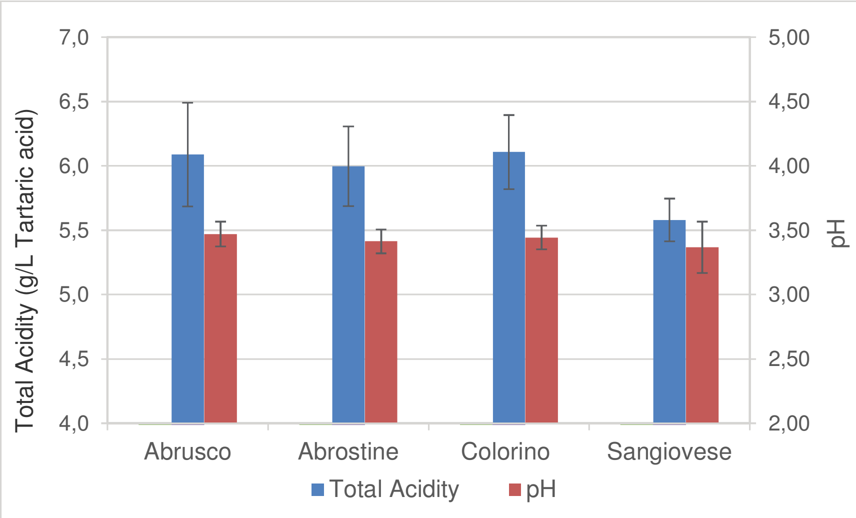

Fig.1 *– Total acidity and pH of the grape samples. Mean values of three years, bars represent ± standard deviations.*

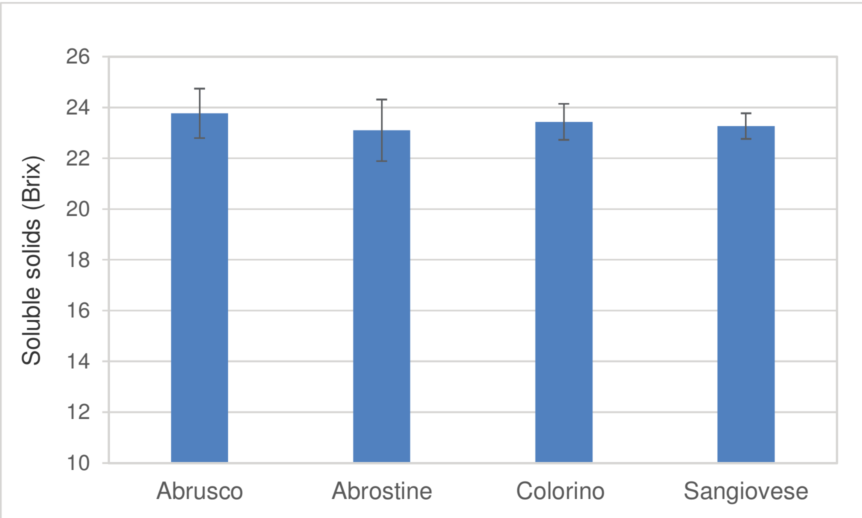

Fig.2 *– Sugar content of the grape samples expressed as soluble solids (Brix). Mean values of three years, bars represent ± standard deviations.*

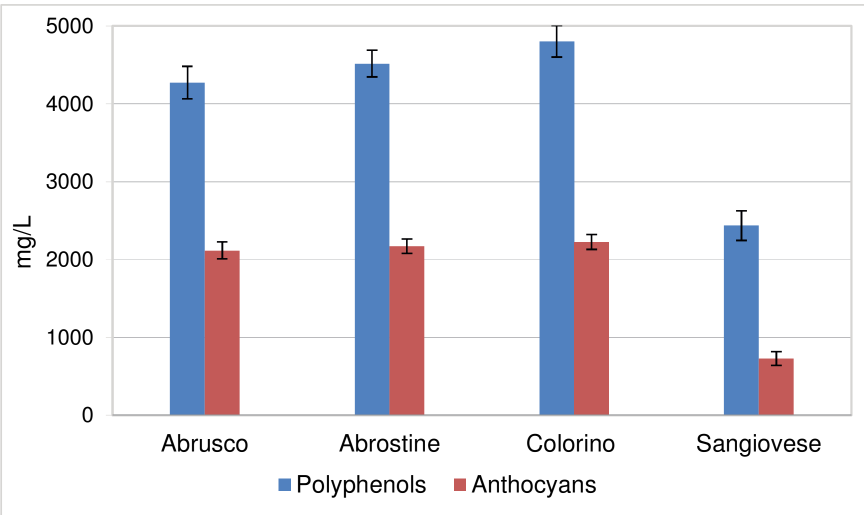

Fig.3 *– Total polyphenols and anthocyanins of grape extracts. Mean values of three years, bars represent ± standard deviations.*

The different composition of the grapes was reflected in the composition of wines. The ethanol content was similar in all the tests; Sangiovese wines had the lowest levels of phenolic compounds, colour intensity and reduced extract. The total acidity resulted higher in the Sangiovese wines than in the other. With regard to the coloured biotypes, all the standard parameters (total acidity, pH, ethanol content, reduced extract) were similar; the Colorino del Valdarno samples were more colourful and had more phenolics than Abrostine and Abrusco wines (Tab.1).

In 2014, microvinifications of Sangiovese grapes blended with Abrostine, Abrusco and Colorino del Valdarno were conducted. In order to assess the effects of the co-fermentation on the phenolic composition and on the colour of the wines, the measured parameters (Real) were compared to the expected values, calculated with weighted means between the data of the original varietal wines (Calc.). The colour intensity and hue, the phenolic and anthocyanins content, measured after five months from the malolactic fermentation, resulted statistically similar. This finding shows that, in this case, the effect of co-fermentation is limited to a simple enrichment of wines, excluding a stimulating effect on the phenomena of copigmentation and colour stabilization (Tab. 2).

Table 1. *Wine compositions. Mean values of three years ± standard deviations. Different letters denote statistically significant differences (95% LSD confidence level).*

	Abrusco	Abrostine	Colorino	Sangiovese
pH	3.50 ± 0.07 [a]	3.53 ± 0.10 [a]	3.46 ± 0.04 [a]	3.29 ± 0.06 [b]
Total Acidity (g/L)	5.54 ± 0.16 [a]	5.55 ± 0.41 [a]	5.52 ± 0.12 [a]	6.16 ± 0.20 [b]
Ethanol (% v/v)	13.57 ± 0.57 [a]	13.28 ± 0.63 [a]	13.33 ± 0.46 [a]	13.22 ± 0.39 [a]
Reduced Extract (g/L)	31.83 ± 2.25 [a]	31.47 ± 2.34 [a]	30.97 ± 1.57 [a]	27.57 ± 1.07 [b]
Anthocyanins (mg/L)	247 ± 19 [b]	260 ± 14 [b]	307 ± 43 [a]	119 ± 14 [c]
Polyphenols (mg/L)	2674 ± 148 [b]	2915 ± 460 [b]	3503 ± 150 [c]	1814 ± 73 [a]
Colour Intensity	17.54 ± 1.16 [b]	18.12 ± 0.27 [b]	20.93 ± 0.93 [c]	7.15 ± 0.46 [a]
Hue	0.68 ± 0.03 [a]	0.63 ± 0.03 [a]	0.65 ± 0.02 [a]	0.75 ± 0.05 [b]

Table 2. *Wine compositions (Vintage 2014). The table shows the results of the analysis performed on the wines five months after the malolactic fermentation, mean values ± standard deviation (Real), and the expected values calculated with weighted means between the data of the original varietal wines (Calc.).*

	Anthocyanins (mg/L)		Polyphenols (mg/L)		Colour Intensity		Hue	
	Calc.	Real	Calc.	Real	Calc.	Real	Calc.	Real
Sangiovese	-	123 ± 12	-	1888 ± 57	-	7.56 ± 0.43	-	0.69 ± 0.03
Abrusco	-	264 ± 16	-	2843 ± 111	-	18.42 ± 0.96	-	0.65 ± 0.03
Abrostine	-	274 ± 21	-	3042 ± 266	-	18.42 ± 0.88	-	0.61 ± 0.03
Colorino	-	348 ± 29	-	3670 ± 110	-	21.90 ± 1.01	-	0.63 ± 0.02
Sangiovese+Abrusco	194	170 ± 17	2366	2439 ± 99	12.99	11.39 ± 0.81	0.67	0.76 ± 0.06
Sangiovese+Abrostine	199	194 ± 14	2465	2253 ± 112	12.99	12.23 ± 0.36	0.65	0.69 ± 0.04
Sangiovese+Colorino	236	207 ± 28	2779	2685 ± 121	14.73	13.36 ± 0.70	0.66	0.72 ± 0.04

The principal component analysis (Fig.4), based on the anthocyanins profiles of three years, clearly separates Colorino del Valdarno grapes (Colo11, Colo12 and Colo13) from the other samples. From the graph emerges that Colorino del Valdarno is distinguished for the highest percentages of malvidin 3-glucoside (Mal3G), malvidin 3-(acetyl)-glucoside (Mal3G_Ac), malvidin 3-(caffeoyl)-glucoside (Mal3G_Ca) and malvidin 3-(p-coumaroyl)-glucoside (Mal3G_Co). Abrusco (Abru11, Abru12, and Abru13) and Abrostine (Abro11, Abro12, and Abro13) cover a large area of the graph, but without separating each other.

In Table 3 the anthocyanins profiles of the Colorino biotypes are reported, the statistically significant differences, marked with different letters, were determined with the analysis of the variance (ANOVA) with a 95% LSD confidence level. The ANOVA establishes that the differences between Colorino del Valdarno and the other biotypes, reported by the PCA, are due to high percentages of all the forms of malvidin 3-glucoside at the expense of the others trisubstituted anthocyanins (delphinidin 3-glucoside and petunidin 3-glucoside) and, furthermore, it shows that Abrusco and Abrostine have identical profiles.

Table 3. *Anthocyanins profiles of grape extracts expressed as relative abundance. Mean values of three years ± standard deviations. Different letters denote statistically significant differences (95% LSD confidence level).*

Compound	Relative abundance (%)		
	Grape variety		
	Abrostine	Abrusco	Colorino
delphinidin 3-glucoside	15,43 ± 1,04 [a]	14,79 ± 0,52 [a]	13,60 ± 0,30 [b]
cyanidin 3-glucoside	2,94 ± 0,41 [a]	2,93 ± 0,08 [a]	2,15 ± 0,41 [b]
petunidin 3-glucoside	13,93 ± 0,70 [a]	13,80 ± 0,39 [a]	11,86 ± 0,29 [b]
peonidin3-glucoside	9,26 ± 0,20 [a]	8,99 ± 0,18 [a]	8,29 ± 1,01 [a]
malvidin 3-glucoside	40,04 ± 1,37 [a]	41,80 ± 1,23 [a]	44,30 ± 2,12 [b]
delphinidin 3-(acetyl)-glucoside	1,92 ± 0,16 [a]	1,74 ± 0,11 [a]	1,53 ± 0,09 [b]
cyanidin 3-(acetyl)-glucoside	0,23 ± 0,05 [a]	0,23 ± 0,06 [a]	0,21 ± 0,03 [a]
petunidin 3-(acetyl)-glucoside	1,68 ± 0,13 [a]	1,50 ± 0,16 [a]	1,48 ± 0,09 [a]
peonidin3-(acetyl)-glucoside	0,70 ± 0,04 [a]	0,65 ± 0,12 [a]	0,70 ± 0,13 [a]
malvidin 3-(acetyl)-glucoside	4,68 ± 0,38 [a]	4,48 ± 0,09 [a]	5,62 ± 0,36 [b]
delphinidin 3-(p-coumaroyl)-glucoside	1,37 ± 0,10 [a]	1,26 ± 0,21 [a]	1,08 ± 0,11 [a]
malvidin 3-(caffeoyl)-glucoside	0,15 ± 0,03 [a]	0,13 ± 0,04 [a]	0,23 ± 0,02 [b]
cyanidin 3-(p-coumaroyl)-glucoside	0,33 ± 0,02 [a]	0,34 ± 0,04 [a]	0,25 ± 0,02 [b]
petunidin 3-(p-coumaroyl)-glucoside	1,04 ± 0,05 [a]	1,01 ± 0,15 [a]	1,05 ± 0,08 [a]
peonidin3-(p-coumaroyl)-glucoside	0,95 ± 0,01 [a]	0,86 ± 0,16 [a]	0,98 ± 0,06 [a]
malvidin 3-(p-coumaroyl)-glucoside	5,29 ± 0,72 [a]	5,44 ± 0,36 [a]	6,63 ± 0,35 [b]

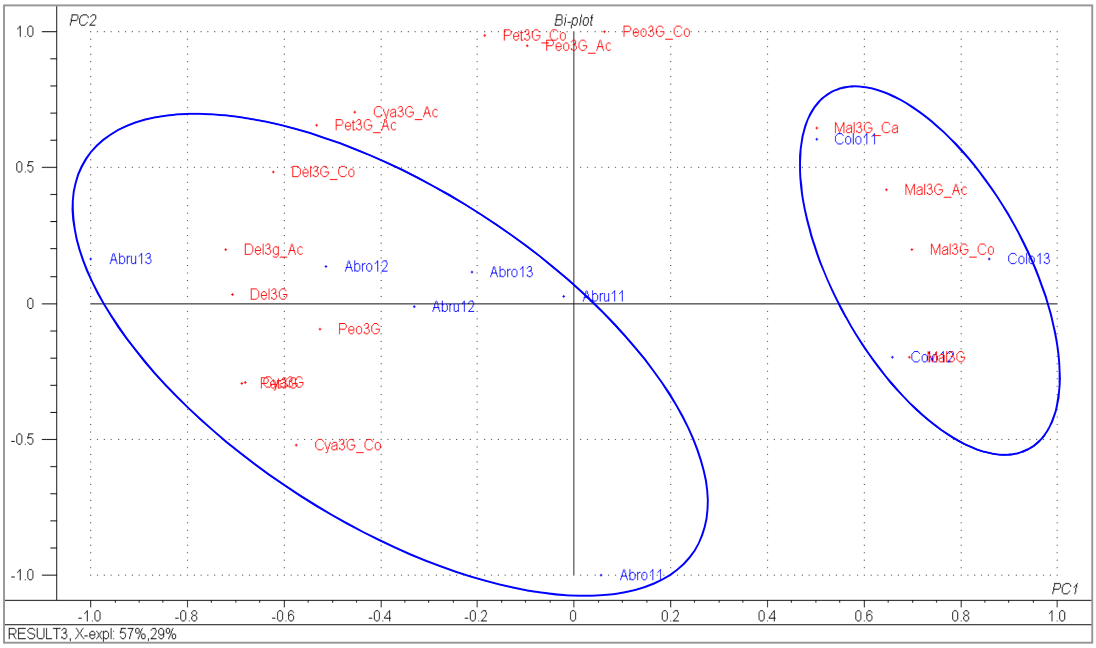

Fig.4. *Bi-plot of the principal component analysis (PCA) of the relative abundances of anthocyanins (loadings) and grape samples (scores).*

Conclusions

In this work the oenological characteristics of three Colorino biotypes (Colorino del Valdarno, Abrusco and Abrostine), as a complement for Sangiovese grapes and wines, were investigated.
The Colorino biotypes grapes, with regard to the standard parameters (sugar content and total acidity), were similar to Sangiovese. The content of phenolic compounds (polyphenols and anthocyanins), as expected, was significantly higher in the coloured grapes and, for this reason, the wines produced from these varieties were distinguished by a higher colour intensity and the phenolic compounds concentration. In general, these grapes are able to bring, during the maceration, greater quantities of solutes (reduced extract).

If vinified in blend with Sangiovese, the role of these varieties is limited to a direct enrichment in phenolic compounds of the wines without inducing additional colour improvement due to copigmentation phenomena and other reactions of stabilization of the phenolic matrix.
Colorino del Valdarno grapes had the highest concentrations of polyphenols. The statistical study of the anthocyanins profiles showed significant differences between Colorino del Valdarno and the other biotypes.

Abrusco and Abrostine can be considered similar: no differences were found in the study of their grapes and wines. This confirms not only what has been observed with genetic methods and image processing, but also the validity of this approach as a powerful tool for the taxonomic study of coloured grapes.

References

A.R.T.E.A. – Agenzia Regionale Toscana per le Erogazioni in Agricoltura, "*Il potenziale viticolo toscano*". https://www.artea.toscana.it/sezioni/documenti/testi/newsletter/ 31-SchedarioViticolo .pdf, (February, 2015).

Bucelli, P. et al. (1995). "*Valutazione di alcuni componenti fenolici in cultivar di vite a bacca nera in Toscana*". *Riv. Vitic. Enol.,* **48***: 39-50.*

Di Stefano, R. et al. (1989). "*Metodi per lo studio dei polifenoli dei vini*". *L'Enotecnico*, **25**: 81-89.

Di Stefano, R. et al. (1997). "*Alcuni aspetti del controllo di qualità nel campo enologico. Lo stato di combinazione degli antociani*". *Annali ISE*, **XXVII**: 105-121.

Glories, Y. (1984). "*La couleur des vins rouges. 2ème partie. Mesure, origine et interprétation*". *Conn. Vigne Vin,* **18**: 253-271.

Mancuso, S. et al. (1998). "*Application of an artificial neural network (ANN) for the identification of grapevine genotypes"*. Vitis, **37**: 27-32.

Mattivi, F. et al. (1990). "*Vitis vinifera - a chemotaxonomic approach: anthocyanins in the skin"*. Vitis, Special Issue: 119-133.

MiPAAF - Ministero per le Politiche Agricole Alimentari e Forestali, "*Registro nazionale delle varietà di vite*", http://catalogoviti.politicheagricole.it/catalogo.php, (February, 2015).

O.I.V. - International Organization of Vine and Wine, "*International Methods of Analysis of Wines and Musts*", http://www.oiv.int/oiv/info/enmethodesinternationalesvin?lang=en, (February, 2015).

Ortega - Regules, A. et al. (2006). "*Anthocyanin fingerprint of grapes: environmental and genetic variations".* J. Sci. Food Agric., **86**: 1460–1467.

Robinson, J. et al. (2013). *"Wine Grapes: A complete guide to 1,368 vine varieties, including their origins and flavours"*. Penguin UK.

Sensi, E. et al. (1996). "*Characterization of genetic biodiversity with Vitis vinifera L. Sangiovese and Colorino genotypes by AFLP and ISTR DNA marker technology"*. Vitis, **35**: 183-188.

Vignani, R. et al. (2008). "*A critical evaluation of SSRs analysis applied to Tuscan grape (Vitis vinifera L.) germplasm"*. Advances in Horticultural Science, **22**: 33-37.

Summary

The Italian viticulture is distinguished by its marked variability in terms of cultivated varieties and clones. In the past, however, many native vines, often not very productive, have been replaced by more profitable varieties. This situation has led close to the extinction of many native cultivars. However, more recently, their oenological potential for the production of typical wines has renewed the interest on these grape vines.

Colorino, as its biotypes Abrostine and Abrusco, is an ancient Tuscan grape variety traditionally used to improve the colour of Sangiovese wines.

In this work some oenological aspects of three biotypes of Colorino preserved in the vineyard collection of CRA-VIC in Arezzo (Italy) are considered.

For three years grape samples of Colorino, Abrostine and Abrusco were analysed to compare their sugar content, total acidity, polyphenols, anthocyanins and anthocyanin profiles to Sangiovese.

In 2014 the same grapes have been vinified and the wines have been analysed to define standard and phenolic parameters.

The results indicated only small differences between the samples, in particular, Colorino showed slightly higher percentages of malvin and p-coumarate anthocyanins.