CHARACTERIZATION OF DIFFERENT CLONE CANDIDATES OF XINOMAVRO ACCORDING TO THEIR PHENOLIC COMPOSITION

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Abstract:
Context and purpose of the study - The aim of this study is the examination of wines of 9 different clones of a Greek grape variety Xinomavro, (XE1, X19, X22, X28, XE2 X30, X31, X35, X36, X37), with regards to their phenolic and anthocyanin content and chemical composition.
Material and methods - Grapes were collected in vintage 2016, from an established Xinomavro vineyard, planted with the nine clones each one represented by fifty plants. The vineyard was established in 2011, with planted material selected according to the corresponding E.U. legislation for vine clone selection.
Grapes were collected at harvest; general chemical analyses of each clone were recorded and the grapes were vinified under the same winemaking protocol and conditions. Monomeric anthocyanins, tannin mean degree of polymerization (mDP), galloylation percentage (%G), percentage of prodelphinidins (% P) and total tannin content, were determined in the produced wines by High Performance Liquid Chromatographer (HPLC) and spectrophotometer.
Results - In most analyses performed an influence of clone selection was observed. Clones XE1, X19, X37, X35 and X31 differentiate from the clones evaluated in parameters crucial for wine quality such as maturity, acidity, anthocyanin, phenolic content and composition. It is therefore a step towards identifying clone characteristics dependent to the viticulture and winemaking needs.
Keywords: mean polymerization degree, Xinomavro, proanthocyanidins, anthocyanins.

1. Introduction

Xinomavro (*Vitis vinifera* L.) is one of the most significant indigenous varieties that are cultivated in Greece, especially in at the central and the west part of Macedonia and also is a part of some P.D.O. wines (Protected Determination of Origin) (Skiada et al, 2010). Xinomavro variety produces black grapes with high acidity , that mature at late September and early October, and the wine ages into a 'complex maturity' (Lambert-Gócs, 1990; Robinson, 1997). The grapes and the wine made from Xinomavro variety is characterized by high acidity, phenolic richness and ageing potential both in bottles and in oak barrels.

Proanthocyanidins are generally oligomers and polymers of flavan-3-ols and they are split into two categories where the first one is procyanidins and the second one the prodelphinidis (Garrido & Borges, 2013; Buckingham et al., 2015). In detail, they are polymers that are being composed of terminal and extension subunits of flavan-3-ols where the typical subunits can be (+)-catechin, (-)-epicatechin (EC), (-)-epicatechin-3-O-gallate (ECG), (+) - gallocatechin, (-)-epigallocatechin (EGC) and epigallocatechin-3-O-gallate (EGCG) (Chira et al., 2009). Partial galloylation of the flavanol 3-hydroxyl group can cause a vast variety of different structured oligomers (Gu et al., 2003). Procyanidins consist of catechin (C) and epicatechin (EC) monomers, and prodelphinidins consist of gallocatechin (GC) and epigallocatechin (EGC) monomers (Buckingham et al., 2015). Furthermore they are the most abundant PA in grapes and wine (He et al., 2008).

Anthocyanins are almost exclusively found in grape skins and only few grape varieties contain anthocyanins into the grape pulp. They are responsible for the red color of wines and have no flavor or other organoleptic property. Anthocyanins predominantly exist as glucosides, which form through the conjugation of the flavonoid component, called anthocyanin with glucose. Their structure, flavylium cation, includes two benzene rings bonded by an unsaturated cationic oxygenated heterocycle, derived from the 2-phenyl-benzopyrylium nucleus (Ribereau-Gyon et al., 2006). The common anthocyanins found in grapes are cyanidin, delphinidin, peonidin, petunidin, and malvidin, with the latter being the most abundant.

2. Material and methods

The experiment was conducted in the wine region of Nemea, in Peloponnese in Greece. The wine samples were red wines, 9 different clones from year 2016. The clones were named E1, X19, X22, X28, X30, X31, X35, X36, and X37. The wine region has pH 8.1 with 1.21 % of organic matter while the slope of the soil is 0-2% and the orientation is from North- South. The *Vitis Vinifera* L. cv. Xinomavro was grafted on American rootstock R110.

Red wine was produced from harvestedXinomavro grapes (20 Kg from each vineyard) in duplicate.After the completion of the alcoholic fermentation the wines were racked and after a month, wines were bottled and stored at 18±2 ℃ in the dark until analyzed.

For the determination of mDP, it has been used an HPLC Jasco (Tokyo, Japan) system, which consists of a PU-2089 plus pump, one infusion valve Rheodyne model 7725i with embedded loop 20 µL and a detector diode array (DAD; Lasco MD-910). The stele that has been used was XTerra RP18 (3.5 µm, 4,6x100mm) from Waters company. The chemical reagents that have been used were catechin, epicatechin, gallic ester of epicatechin, epigallocatechin and gallic ester of epigallocatechin for the formation of the standard curves, methanol, acetic acid, phloroglucinol, ascorbic acid, hydrochloric acid and sodium acetate, they were from Sigma-Aldrich. For the deterrmination of the anthocyanin content, the stele that has been used was Pinnacle II C_{18} (5µm, 4.6x250mm) from Restek company. The chemical reagents that have been used were malvidin, for the formation of the standard curves, and also methanol and formic acid for the determination of anthocyanins, were bought from Sigma-Aldrich.

Anthocyanin analysis for the determination of monomeric anthocyanins was performed according to Kyraleou et al. (2015). Briefly, a Restek pinnacle II C_{18} (250 x 4.6 mm, 5 µm) column was used at a flow rate of 1 mL/min, using a 10 µL injection volume, detection at 520 nm. Identification was based on comparing retention times and UV spectra of the peaks detected with those of original compounds. Malvidin-3-Oacetylmonoglucoside (MlvAcc) and malvidin-3-(6-Op-coumaroyl) monoglucoside (MlvCoum) were tentatively identified based on previous observations (Kallithraka et al., 2005). Results were expressed as mg/L and all analyses were performed in duplicate.

Data were subjected to one-way analysis of variance (ANOVA), using Statistica V.7 software (Statsoft Inc., Tulsa, OK). Comparison of mean values was performed using Tukey's HSD test when samples were significantly different by ANOVA ($p < 0.05$) in a 95% significance level.

3. Results and discussion

Classical analyses were performed on wines and the results are summarized in Table 1. Alcohol level (% vol) values ranged from 11.7 to 13.1 % vol., titratable acidity from 6.0 to 6.7 (gr/lt as tartaric acid), pH values ranged from 3.28 to 3.37, malic acid content from 1.3 to 1.9 gr/lt and tartaric acid content from 2.5 to 3.0 gr/lt. Finally, color density values ranged from 6.9 to 9.1, anthocyanin content from 131.6 to 185.0 mg/lt and phenolic index ranged from 35.1 to 43.1 absorbance units (A280 nm).

Clones XE1 and X19 presented the higher alcohol content while clone X35 and X37 presented the highest anthocyanin content, with the latter presenting the higher color intensity. These clones (in addition clone X22) were also the clones with highest phenolic content. In contrast X31 presented the lowest alcohol and pH level, anthocyanin and phenolic content and the highest malic acid content, characteristics of wines produced from unripe grapes.

Mean degree of polymerization (mDp) values, percentage of galloylation (%G) and percentage of prodelphinidins (%P) were also calculated and presented in Table 2. MDp values ranged between 1.68 (X30) to 1.85 (X36), %G ranged from 1.08 (X37) to 1.38 (X37) and %P ranged between 41.08 (X19) to 49.69% (X37). In accordance to the findings presented in Table 1, X37, X22, X35 presented the highest catechin equivalent values (mg/lt).

The mDP values in the present study, were lower than the values reported by other studies, among them for Cabernet Sauvignon and Merlot by Chira et al. (2011), Tempanillo, Monastrell, Syrah by Busse-Valverde et al., (2012) and Quijada-Morin et al., (2012), Graciano and Tempanillo by Monagas et al., (2003). Similar mDp values were only reported in wines from Agiorgitiko (1.38 to 2.48) by Petropoulos et al., (2017). In general, %G values were lower than the respective values reported for international wines and similar to %P values (Chira et al., 2011, 2012; Quijada-Morin et al., 2012). Comparing our results in Xinomavro with Agiorgitiko (Petropoulos et al., 2017), we reported higher %G and lower %P values.

The relation between mDP values and wine astringency is well documented but not all studies are in agreement. Chira et al., (2011) and Chira et al. (2012) demonstrated a positive correlation while no significant correlation was reported by Quijada-Morin et al., (2012) and Wollman and Hofmann (2013). Controversies have been reported in literature regarding the %G values. Some studies correlate positively %G values to perceived astringency (Chira et al., 2011; Curco et al., 2014) while others report absence (Wollmann and Hofmann, 2013; Kyraleou et al., 2015).

Figure 1, shows the composition of wine proanthocyanidins, obtained after phloroglusinolysis. EGC was the predominant wine subunit ranging from 76 to 81%, followed by C (6-8%), EC (7-11%) and ECG (2-2.5%). There is a positive correlation related between EC concentration and perceived astringency (Quijada-Morin et al., 2012, 2014) while a decrease of perceived astringency is reported with elevated EGC concentration (Vidal et al., 2003). The percentage of subunits was not influenced by clone selection.

The anthocyanin composition of the clones examined is presented in Table 3. All examined clones presented low anthocyanin content in relation to international and Greek grape varieties in agreement with literature (Kallithraka et al., 2005; Makris et al., 2006; Kyraleou et al., 2015) Clone X35 presented the higher total anthocyanin content (196 mg/lt) while X31presented the lowest (100 mg/lt), not only in total values but also in all individual anthocyanin values determined.

4. Conclusions.

This study is a first attempt to evaluate and characterize Xinomavro clones. Our results showed an impact of clone selection on wine compositional characteristics with differentiations in phenolic composition, colorization and maturation. However, the information provided in this study is a preliminary trial on Xinomavro clonal evaluation and more data from more vintages need to be examined including sensory evaluation.

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Table 1: Compositional factors determined in the wine clones.

	% vol.	T.A.	pH	Malic acid	Tartaric acid	Color Density	Anthocyanins	Phenolics*
XE1	13,1	6,7	3,31	1,4	3,0	7,8	153,0	43.1
X19	13,1	6,3	3,33	1,3	2,8	8,2	171,5	39.3
X22	12,1	6,3	3,34	1,6	2,7	8,8	170,1	41.2
X28	12,0	6,4	3,32	1,6	2,7	6,9	156,5	36.4
X30	12,9	6,3	3,37	1,5	2,7	6,8	145,5	38.7
X31	11,5	6,5	3,28	1,9	2,7	8,1	131,6	35.3
X35	12,3	6,0	3,41	1,4	2,4	7,2	185,0	37.3
X36	11,7	6,3	3,35	1,9	2,5	7,9	161,9	35.1
X37	12,6	6,5	3,36	1,8	2,7	9,1	184,2	41.0

Alcohol content (%.vol) is expressed as %v/v, titratable acidity (T.A.) is expressed in gr tartaric acid/lt, malic and tartaric acid are expressed in g/lt, anthocyanins are expresses in mg/lt.

*absorbance at 280 nm.

Table 2: Analytical tannin content of the clones examined (vintage 2016). Analyses performed in duplicates. Results are given as mean±std.err.

Clones	BSA*	mDP	%G	%P
XE1	198±0.01	1.82±0.02	1.08±0.09	45.91±0.22
X19	165±0.16	1.78±0.02	1.37±0.07	41.08±0.25
X22	245±0.03	1.82±0.02	1.24±0.05	42.43±0.26
X28	203±0.22	1.73±0.00	1.18±0.00	45.38±0.00
X30	193±0.02	1.68±0.02	1.18±0.02	44.55±0.44
X31	193±0.19	1.82±0.00	1.20±0.03	46.56±0.62
X35	205±0.01	1.81±0.04	1.27±0.02	44.94±0.68
X36	152±0.01	1.85±0.00	1.21±0.07	44.65±0.37
X37	230±0.02	1.73±0.01	1.38±0.04	49.69±0.45

*Values of BSA are expressed as mg/lt catechin measured after precipitation with bovine serum albumin, preformed in duplicate.

Figure 1: Percentage (%) of proanthocyanidins subunits determined in Xinomavro wines from vintage 2016. Values are the means of duplicate determinations.

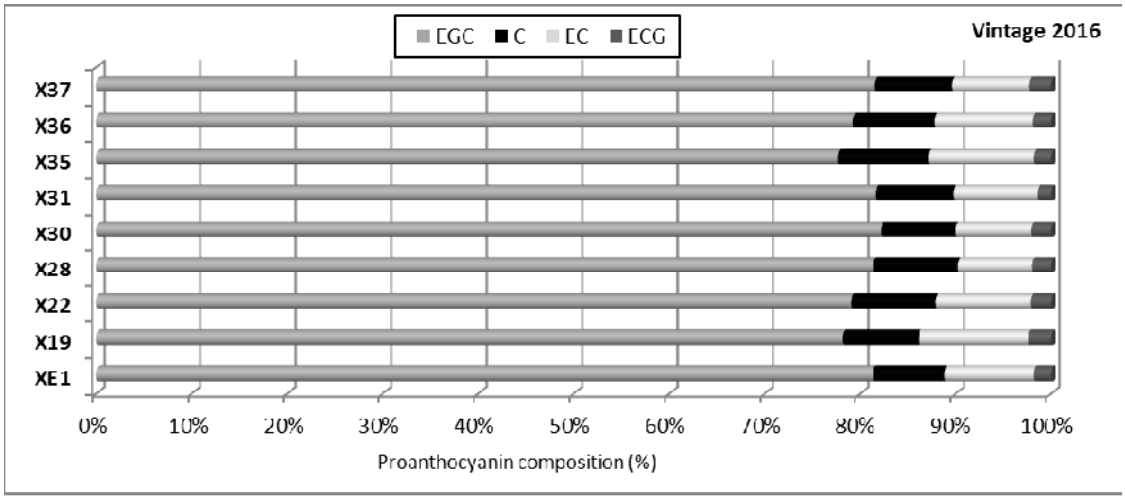

Table 3: Analytical anthocyanin composition of the clones examined for the year 2016. Analyses performed in duplicates. Results are given as mean±std.err.

Clones	Anthocyanin content (mg/L)*							
	Dp	Cy	Pt	Pn	Mlv	MlvAcc	MlvCoum	Total
E1	1.83±0.00	1.94±0.00	4.56±0.00	2.45±0.00	96.79±0.00	11.49±0.00	14.94±0.00	134±0.01
X19	1.62±0.04	2.23±0.05	5.75±0.01	3.14±0.03	113.92±0.58	13.31±0.06	21.82±0.10	162±0.86
X22	1.66±0.07	2.29±0.02	5.27±0.03	3.01±0.03	107.27±0.03	12.15±0.05	18.14±0.06	150±0.18
X28	1.62±0.01	2.14±0.02	5.39±0.09	4.34±0.00	106.36±0.45	12.90±0.09	21.01±0.01	154±0.48
X30	3.23±0.02	1.57±0.01	4.12±0.01	2.15±0.01	96.12±0.31	11.85±0.17	15.81±0.13	135±0.61
X31	0.95±0.02	1.14±0.05	3.36±0.05	1.88±0.01	71.76±0.22	8.91±0.02	11.55±0.10	100±0.36
X35	1.53±0.00	1.40±0.05	6.26±0.09	3.70±0.01	140.9±0.13	16.05±0.04	25.67±0.00	196±0.05
X36	1.37±0.02	1.51±0.03	4.31±0.03	2.38±0.01	96.09±0.24	11.54±0.09	15.91±0.16	133±0.52
X37	1.68±0.01	2.30±0.02	4.55±0.08	4.62±0.03	105.66±0.16	12.79±0.07	20.73±0.08	152±0.14

*HPLC determination of anthocyanins for each clone was performed in duplicate, Results are given as mean±std.err.