Mean polymerization degree of tannins of grape seeds and skins from *Vitis vinifera* var. Xinomavro: Effect of trellising system

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Abstract

Because of their unique varietal diversity, Greek grapes may vary largely in their tannin mean polymerization degree (mDP), a parameter that is closely related with the overall quality of the produced wines. Since this parameter is mostly unexploited regarding the native Greek grape varieties, the aim of the present study was to provide new insights into the mDP of skin and seed tannins of *Vitis vinifera* var. Xinomavro. It was also of interest to study the possible effects of three trellising systems Guyot, Royat and Lyre on the grape phenolic composition and mDP.

For this reason the percentage of galloylation (%G) and the concentrations of (+)-catechin, (-)-epicatechin and procyanidins B1, B2 and C1 were determined by HPLC and LC/MS. The results showed that the grapes seeds derived from the Lyre system were characterized by a higher mDP of tannins in comparison with the grape seeds from the other two training systems studied. In contrast, the skins of grapes from the Lyre system showed the lower value of mDP. However, as far as the monomers catechin and epicatechin are concerned, significant differences were observed among the different seed extracts.

Introduction

Xinomavro is the main red grape variety cultivated in North Greece. Grapes and wines produced from this variety characterized by high acidity and phenolic richness resulting to long ageing potential. Fresh wines usually contain hard tannins and have a unique aromatic character composed of fruity and vegetal aromas. The cultivation of Xinomavro is difficult and is a challenge for the winemakers. To improve the quality of Xinomavro different cultivation and vinification techniques were developed. One of them, which was investigated in this work, was the effect of trellising system on phenolic composition.

Trellising system is the arranging of the vine in space. It affects the relation of leaves to fruits, the fruit zone microclimatethe maturity of grapes and as a consequence the phenolic and anthocyanin composition of grapes (*Smart & Robinson 1991, Downey, et al. 2004, González-Neves et al. 2004, Orlandini et al. 2008, Río Segade, et al. 2009, Mota et al. 2011*)

In red varieties phenolic compounds are an important group which affects the quality of the wine. Proanthocyanidins or condensed tannins are polymers composed of flavan-3ols subunits. They are located in grape skins and seeds and are responsible for the stabilization of the colour and the sensory characteristics of the wines due to their astringent and bitter properties (*Ribereau-Gayon et al. 1999, Chira et al. 2009, Sun et al. 2013*). According to studies, the intensity of astringency is related to their

degree of polymerization and increases with molecular size at least up to DP=6 and then decreases because become or no longer soluble or too bulky to bind to the proteins (*Ribereau-Gayon et al. 1999, Sun et al. 2013*). In addition, molecular size of proanthocyanidins affects their bitterness and so monomers are more bitter than polymers (*Peleg et al. 1999, Chira et al. 2009*). Organized polymerization produces polymerized procyanidins that are increasingly reactive with proteins and, therefore, have an increasingly astringent character. This development continues up to a limit of 8 or 10 flavanol units. On the contrary, polymerization mediated by ethanol softens the flavour.

For characterising the tannin structure extraction from the grapes by using specific solvents should be achieved firstly and then further fractionation based on their molecular size would be necessary. The difficulty to isolate individual tannins from grapes and to analyse them, leads to the characterisation of condensed tannins by depolymerisation. Treatment of condensed tannins with acid, in the presence of a nucleophile such as phloroglucinol (*Prieur et al. 1994, Souquet et al. 1996*) allows the subunit profile to be analysed by HPLC and the calculation of the average molecular mass, expressed as mean degree of polymerisation (mDP).

Extensive research has been contacted in order to investigate tannin composition and structure using the depolymerisation method. The results showed that grape tannins derived from the skin and seeds differ in their length, their subunit composition and sensory properties (*Peleg et al. 1999*) Seed tannins are shorter, with a lower mDP, and have a higher percentage of subunits bearing gallic acid esters, which is expressed as degree of galloylation (%G) (*Prieur et al. 1994*). Skin tannins are generally larger with a higher mDP (*Souquet et al. 1996*).

The variety effect on proanthocyanidin composition and mDP of skins and seeds have been demonstrated in previous studies (*Chira et al. 2009, Mattivi et al. 2009*). The aim of the present work was to investigate the mDP of seed and skin tannins of a native Greek variety (Xinomavro) and to examine the possible effect of three different trellising systems Guyot, Royat and Lyre on this parameter.

Materials and methods

Experimental conditions

The experimental vineyard was situated in Northern Greece, in the region of Naoussa, in Kyr- Yanni Estate , planted with *Vitis vinifera* L. cv. Xinomavro. Samples were collected from three different trellising systems Guyot, Lyre and Royat, at technological maturity in September of 2010 in triplicates of each treatment and kept frozen until analysis. Vinification of red wine was carried out separately for each treatment.

Materials and methods

Seeds and skins of 150 berries were removed by hand from grapes, lyophilised for 2 days and stored at -20 ^{o}C. The frozen seeds and skins were finally ground to obtain powder. (*Chira et al. 2009, Lorrain et al. 2011*)

The extractions of skin and seed tannins were carried out according to previous methods (*Chira et al. 2009, Lorrain et al. 2011*). A 3 g portion of the obtained powder was extracted using 25mL of acetone/ water (80:20, v/v) for 3 h and 25 mL of methanol/water (60:40, v/v) for 2.5 h. The centrifugal supernatants were combined and evaporated under reduced pressure at 30 ^{o}C to remove organic solvents; the residue was dissolved in water and lyophilised to obtain a crude tannin extract. Crude extracts were solubilised in 5% of ethanol and extracted three times with chloroform to remove lipophilic material. Then the aqueous phase was extracted three times with ethyl acetate. Organic and aqueous fractions were collected, concentrated and lyophilised to obtain dry powder. The organic fraction contains monomeric and oligomeric proathocyanidins and the aqueous contains polymeric tannins.

The organic fraction for seeds and skins were analyzed by HPLC–UV according to *Kallithraka et al*. (2006) for the determination of (+)-catechin, (-)-epicatechin and procyanidins B1, B2 and C1. Identification was based on comparing retention times of the peaks detected with those of original compounds, and on UV–vis on-line spectral data. Quantification was performed by establishing calibration curves for each compound determined, using the standards. Results were expressed as μg per g dry weight. All analysis performed in triplicate.

Furthermore, tannin mean polymerization degree (mDP) and percentage of galloylation (%G) were determined in both organic and aqueous phase for seeds and skins extracts. Tannin extracts were resolubilised in methanol and reacted with phloroglucinol solution (50 g/L phloroglucinol, 10g/L ascorbic acid, 0,1N HCl, in methanol) for 20 min at 50 ºC and then the reaction stopped with the addition of aqueous sodium acetate (40mM). LC-MS and HPLC analyses of these solutions were performed for the identification and quantification of phloroglucinol adducts and terminal units (*Chira et al. 2009, Lorrain et al. 2011*, *Kennedy et al. 2001, Drinkine et al.2007b*). All analysis performed in triplicate.

Statistics

Data were subjected to one-way analysis of variance (ANOVA), of Statistica V.7 Software (Statsoft InC., Tulsa, OK). Comparison of mean values were performed using Tukey's HSD test when samples were significantly different after ANOVA ($p < 0.05$).

Results

As reported in *Figure 1*, oligomeric fractions showed higher mDP in skins with values 2,3 to 3,3 than in seeds (values 1,9 – 2,1). In addition, the samples from the trellising system Royat presented significantly higher value of mDP in skins, while Lyre had the higher mDP in seeds. There was no statistical difference in mDP values of seeds among the two vertical shooting trellises Guyot and Royat. Significant differences were found in mDP of polymeric fractions from seeds and skins among the three trellising systems. In Royat system was observed the higher mDP in both seeds and skins (*Figure 2*).

Figures 3 and 4 show the percentage of galloylation (%G) of skins and seeds from the three trellising systems for both oligomeric and polymeric fractions. Oligomeric fractions showed higher values of %G than polymeric fractions. Also, there were no significant differences in the %G of oligomeric fractions for each treatment. However, it was observed significant difference of %G for the polymeric tannin fractions. Royat showed the lowest %G for seeds and skins, compared to the other two trellising systems.

Trellising systems (Royat, Lyre, Guyot) affected the phenolic composition of skin and seed tannin extracts of Xinomavro variety (*Table 1&2*). According to the results, Royat system showed higher values of monomeric proanthocyanidins, especially catechin, in skin extracts but lower values in seed extracts. There were no significant differences in oligomeric proanthocyanidins of skin extracts, while there were not detected any gallic acid esters in the same samples. Significant differences were found between the concentrations of gallic acid esters in seed extracts with Lyre showing the lower sum. In Guyot system was observed the higher content of catechin, proanthocyanidin B1 and C1. In addition, , it can be observed that seeds were almost 100 times richer in oligomeric proanthocyanidins as compared to skins.

Conclusions

According to the results, Xinomavro has lower mDP compared to the well studied international varieties such as Merlot and Cabernet Sauvignon (*Chira et al. 2009, Lorrain et al. 2011*). Thus probably leading to more bitter/astringent wines in general. Furthermore, the trellising system possibly affects both the mDP, %G of polymeric fractions and the mDP of oligomeric fractions. In addition, it seems that

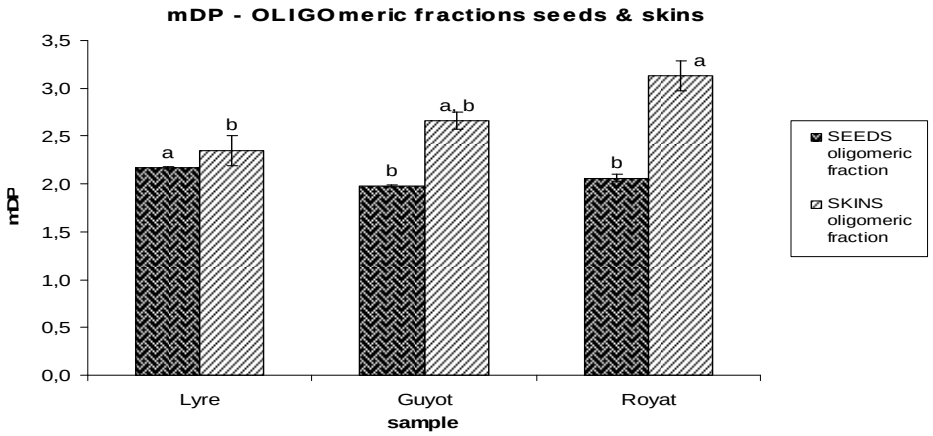
trellising system has an effect on the monomeric phenols in skins and seeds. Grapes from Lyre system showed the highest mDP and total concentration of gallic acid esters both in seed oligomeric fraction and in seed and skin polymeric fractions. Royat presented significantly higher value of mDP in skins and the lowest %G for seeds and skins, compared to the other two trellising systems.

Trellising system did not affect %G in oligomeric fraction of skins and seeds and concentration of total oligomeric phenols in seeds and skins. It was observed that %G of polymeric fractions was lower than that of oligomeric fractions, while the opposite was true for mDP.

In order to gain insights on the influence of the trellising system to the quality of the grapes a second year of experiments will be adequate in order to confirm the effects in different vintages.

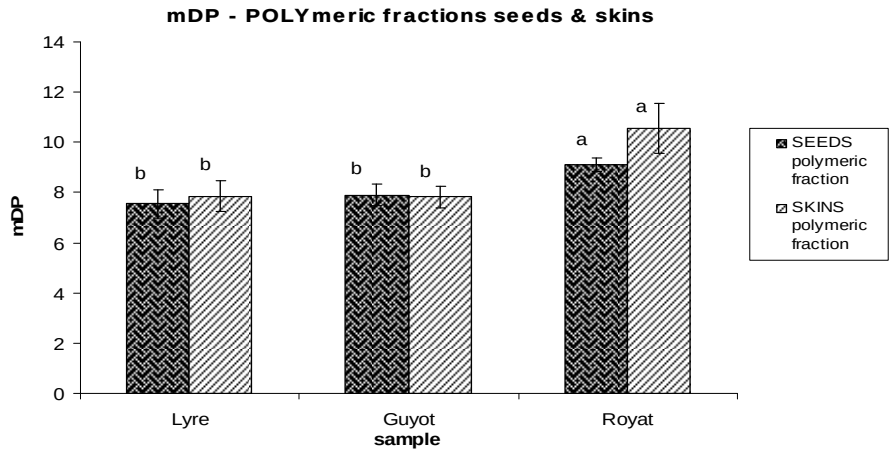
Acknowledgments

Samples for this research were provided by Kir – Yianni Estate, Naoussa



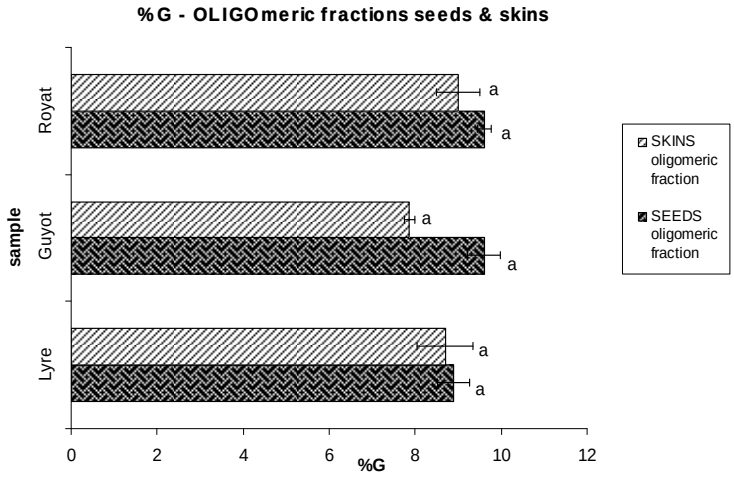
values with different letter within seeds or skins are significantly different (Tukey's test, p < 0,05)

Figure 1: mean degree of polymerization of oligomeric fractions from seeds and skins



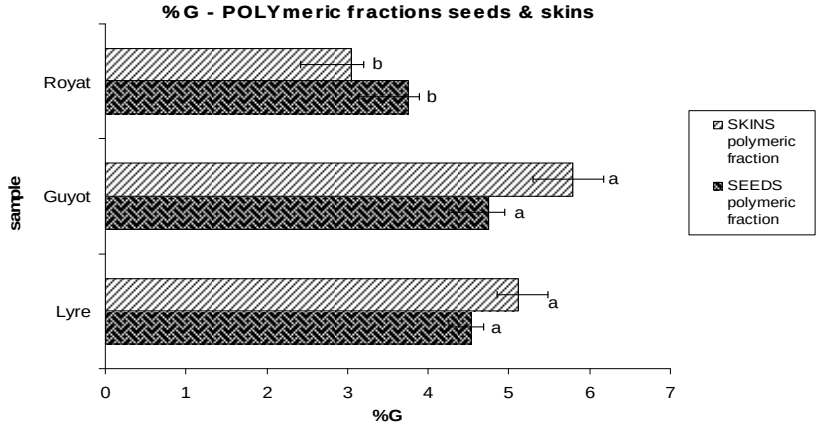
values with different letter within seeds or skins are significantly different (Tukey's test, p < 0,05)

Figure 2: mean degree of polymerization of polymeric fractions from seeds and skins



values with different letter within seeds or skins are significantly different (Tukey's test, p < 0,05)

Figure 3: percentage of galloylation of oligomeric fractions from seeds and skins



values with different letter within seeds or skins are significantly different (Tukey's test, p < 0,05)

Figure 4: percentage of galloylation of polymeric fractions from seeds and skins

Table 1: concentrations of seed phenolic compounds from the three trellising systems in mg/g dry weigh

	Lyre	Royat	Guyot
gallic acid	0,16 ± 0,01 [a]	0,19 ± 0,01 [a]	0,19 ± 0,01 [a]
catechin	6,93 ± 0,62 [a,b]	5,30 ± 0,24 [b]	7,29 ± 0,23 [a]
epicatechin	4,56 ± 0,41 [a]	4,06 ± 0,21 [a]	4,95 ± 0,27 [a]
B1	1,18 ± 0,08 [a,b]	0,98 ± 0,02 [b]	1,26 ± 0,05 [a]
B2	1,28 ± 0,12 [a]	1,21 ± 0,02 [a]	1,33 ± 0,05 [a]
C1	1,25 ± 0,16 [a]	0,99 ± 0,11 [a]	1,08 ± 0,10 [a]
ECG	0,09 ± 0,01 [a]	0,07 ± 0,00 [b]	0,08 ± 0,00 [a]
EGCG	0,76 ± 0,06 [b]	1,03 ± 0,01 [a]	1,06 ± 0,03 [a]

values with different letter within each row are significantly different (Tukey's test, $p < 0,05$)

Table 2: concentrations of skin phenolic compounds from the three trellising systems in mg/g dry weigh

	Lyre	Royat	Guyot
gallic acid	0,031 ± 0,004 [a]	0,020 ± 0,008 [a]	0,019 ± 0,003 [a]
catechin	0,051 ± 0,005 [b]	0,117 ± 0,047 [a]	0,041 ± 0,004 [b]
epicatechin	0,030 ± 0,004 [a]	0,056 ± 0,020 [a]	0,008 ± 0,003 [b]
B1	0,012 ± 0,001 [a]	0,034 ± 0,013 [a]	0,042 ± 0,007 [a]
B2	0,022 ± 0,004 [a]	0,037 ± 0,014 [a]	0,010 ± 0,004 [a]
C1	0,006 ± 0,001 [a]	0,009 ± 0,003 [a]	0,004 ± 0,001 [a]
ECG	nd	nd	nd
EGCG	nd	nd	nd

values with different letter within each row are significantly different (Tukey's test, $p < 0,05$)

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