EFFECT OF VINE TRAINING SYSTEM ON THE PHENOLIC COMPOSITION OF RED GRAPES (*VITIS VINIFERA* L. CV. XINOMAVRO)

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Abstract

Aims: In order to investigate the effect of vine training system on grape phenolic composition, a divided canopy system (Lyre) and a vertical shoot positioned trellis with two different pruning systems (Royat and Guyot) were applied in a commercial vineyard of cv. Xinomavro (*Vitis vinifera* L.).

Methods and results: Monomeric anthocyanins, flavan-3-ol monomers and oligomers, tannin mean degree of polymerization (mDP), galloylation percentage (%G), and extension and terminal subunit structure were determined in seeds and skins by HPLC and LC/MS. Total phenolics, anthocyanin content, tannin concentration and antioxidant activity were measured in whole berries. Grapes and wines of the Lyre system were characterized by increased concentration of total and individual anthocyanins and total phenols. Individual flavan-3-ol monomers and oligomers were highest in Royat grape skins and Guyot seeds. Concerning proanthocyanidin structure, Royat grapes had significantly higher mDP and lower %G in skin and seed polymeric tannin fractions, compared to the other two systems, possibly leading to a lower grape astringency potential.

Conclusion: Training system may affect grape anthocyanin concentration, total flavan-3-ol content as well as skin and seed proanthocyanidin structure.

Significance and impact of the study: The results of the present study could be of high importance to both grape growers and winemakers. Xinomavro grapes are rather poor in anthocyanins while being characterized by dry and astringent tannins. By adopting Lyre as a training system, higher anthocyanin contents could be achieved in grapes, resulting in wines with more attractive colour when adopting a longer pre-fermentation skin contact process. On the contrary, grapes of the Royat system might be more appropriate to produce full-bodied wines with higher ageing potential, due to their more appropriate tannin structure, by using longer maceration periods.

Key words: grapes, training system, anthocyanins, proanthocyanidins, mean degree of polymerization, percentage of galloylation

Résumé

Objectifs : Afin d'étudier l'effet du système de conduite de la vigne sur la composition phénolique du raisin, un système de division du feuillage (Lyre) et un système de positionnement vertical des rameaux avec deux tailles différentes (cordon Royat et Guyot) ont été appliqués dans un vignoble commercial du cépage Xinomavro (*Vitis vinifera* L.).

Méthodes et résultats : La concentration des monomères des anthocyanes et des monomères et oligomères des flavan-3-ols, ainsi que le degré moyen de polymérisation (mDP), le pourcentage de galloylation (%G) et la structure des unités terminales et d'extension des tannins ont été déterminés dans les pépins et les pellicules des raisins par HPLC et LC/MS. Les concentrations en composés phénoliques, anthocyanes et tanins totaux de la baie et la capacité antioxydante des raisins ont aussi été mesurées. Les baies et les vins issues des vignes conduites en Lyre ont été caractérisées par une teneur plus élevée en anthocyanes et en phénols totaux. Les pellicules des baies du cordon Royat et les pépins de celles issues du Guyot contenaient davantage de monomères et d'oligomères de flavan-3-ols. Concernant la structure des proanthocyanidines, les baies du Royat ont présenté dans leur fraction tannique un mDP supérieur et un %G inférieur par rapport aux autres systèmes, menant probablement à un potentiel astringent des raisins moins important.

Conclusion : Le système de conduite peut influencer la teneur en anthocyanes et en tanins des baies de raisins de Xinomavro, ainsi que leur structure tannique.

Signification et impact de l'étude : Les résultats de cette étude pourraient avoir une importance significative pour les viticulteurs et les producteurs de vins. Les raisins de Xinomavro sont relativement pauvres en anthocyanes, tout en possédant des tanins secs et astringents. En adoptant la Lyre, les producteurs de Xinomavro pourraient obtenir des raisins et des vins plus riches en couleur, et les valoriser par l'application d'une macération préfermentaire prolongée. Au contraire, les raisins issus du cordon Royat pourraient être plus appropriés pour la production des vins structurés, avec un potentiel élevé de vieillissement, en raison de leur structure tannique plus favorable.

Mots clés : raisins, système de conduite, anthocyanes, proanthocyanidines, degré moyen de polymérisation, pourcentage de galloylation

manuscript received 28th May 2014 - revised manuscript received 14th December 2014

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J. Int. Sci. Vigne Vin, 2015, **49**, 71-84

INTRODUCTION

Phenolic compounds represent one of the most important groups of compounds for red wine as they affect directly wine quality. Proanthocyanidins (condensed tannins) are polymers composed of flavan-3-ol 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 (Ribéreau-Gayon *et al.*, 1999; Chira *et al.*, 2009; Sun *et al.*, 2013).

The level of astringency is related not only to the concentration of tannins but also to their degree of polymerization. Astringency increases with increasing tannin size, at least up to 8 or 10 flavanol units (polymerized procyanidins are increasingly reactive with proteins and, therefore, have an increasingly astringent character), and then decreases since they become either no longer soluble or too bulky to bind with the proteins (Chira *et al.*, 2009; Sun *et al.*, 2013). On the contrary, tannin polymerization mediated by ethanol softens their flavour (Chira *et al.*, 2012). In addition, the molecular size of proanthocyanidins affects their bitterness, since monomers are more bitter than polymers (Peleg *et al.*, 1999; Ribéreau-Gayon *et al.*, 1999). Astringency is also reported to increase with the degree of tannin galloylation (Cosme *et al.*, 2009; Chira *et al.*, 2011; Ćurko *et al.*, 2014). Galloylation has been shown to increase tannin interactions with various proteins, suggesting that it could be responsible for increased astringent sensation (Cheynier *et al.*, 1997).

In order to characterize tannin structure, extraction from grapes with the use of specific solvents should be applied, following tannin fractionation in accordance to their molecular size. Since tannin isolation and individual analysis are rather difficult procedures, depolymerization is often employed to facilitate their characterization. Treatment of condensed tannins with acid, in the presence of a nucleophile such as phloroglucinol (Prieur *et al.*, 1994; Souquet *et al.*, 1996), allows subunit profiling by HPLC and calculation of the average molecular mass, expressed as mean degree of polymerization (mDP).

Extensive research has been conducted in order to determine tannin composition and structure using the depolymerization method. The results showed that grape tannins derived from skins and seeds vary in length, subunit composition and sensory properties (Peleg *et al.*, 1999). Seed tannins are shorter, with a lower mDP, and display a higher percentage of subunits bearing gallic acid esters, which is expressed as percentage of galloylation (%G) (Prieur *et al.*, 1994). Skin tannins are generally larger with a higher mDP (Souquet *et al.*, 1996). However, seed and skin proanthocyanidins were found to be equally astringent when tasted at the same concentration in a wine or buffer medium, despite their rather large compositional differences (Brossaud *et al.*, 2001).

Xinomavro (*Vitis vinifera* L.) is an indigenous Greek red grape variety cultivated almost exclusively in Northern Greece vineyards producing Protected Denomination of Origin (P.D.O.) red wines. Grapes and wines produced from this variety are characterized by high acidity and phenolic richness resulting in a long ageing potential of the wines. However, wines made from Xinomavro grapes are particularly poor in anthocyanins despite being rich in skin and seed flavan-3-ols (Kallithraka *et al.*, 2006). Therefore, young wines are typically pale in colour with «thin» tannins, making Xinomavro vinification a challenging task for winemakers. Although grape and wine tannin composition is of great technological significance for red wines, for the major wine grape varieties cultivated in Greece, such as Xinomavro, tannin mDP has not been examined, although some investigations have shown that grape variety genotype influences proanthocyanidin composition and mDP of skins and seeds (Chira *et al.*, 2009; Cosme *et al.*, 2009; Mattivi *et al.*, 2009).

Several strategies for manipulating the phenolic composition of red wines have been attempted either during grape growing or wine making. The choice of the vine training system, i.e. the arrangement of vine organs in space with the use of an adapted trellis, is one the major techniques applied by grape growers to influence source-sink relationships and fruit-zone microclimate, with the aim to control grape maturity and composition (González-Neves *et al.*, 2004; Reynolds *et al.*, 2004; Pérez Lamela *et al.*, 2007; Reynolds *et al.*, 2009; Río Segade *et al.*, 2009; Mota *et al.*, 2011). In previous studies it was reported that the phenolic composition of grapes and wines differed between vertical shoot positioned (VSP) and divided canopy systems (Katerji *et al.*, 1994; González-Neves *et al.*, 2004; Pérez Lamela *et al.*, 2007; Río Segade *et al.*, 2009) and also among VSP systems (Río Segade *et al.*, 2009). However, to the best of our knowledge, there have been no studies concerning the effect of training system on the structural characteristics of skin and seed proanthocyanidins such as mDP and %G.

Therefore, the aim of the present work was to investigate the effect of training system on the phenolic composition of Xinomavro grapes and wines. For this purpose, a divided canopy system (Lyre) and a vertical shoot positioned trellis with two different pruning systems (Royat and Guyot) were studied in the vineyard. The phenolic characterization of Xinomavro grapes was performed with respect to the following three analytical parameters: anthocyanin content, tannin composition and proanthocyanidin structure.

MATERIALS AND METHODS

1. Chemicals

Ethyl acetate, chloroform, methanol, ethanol, acetone, sodium metabisulfite, sodium carbonate, phloroglucinol, L(+)-tartaric acid, (+)-catechin, L-ascorbic acid, hydrochloric acid (37%), sodium hydroxide, acetic acid, and 2,2-diphenyl-b-picrylhydrazyl (DPPH) radical were purchased from Sigma Aldrich (Saint Louis, USA). Bovine serum albumin (BSA, fraction V) was obtained from Applichem. (-)-Epicatechin, (-)-epigallocatechin, (-)-epicatechin-3-*O*-gallate, and proanthocyanidins B1, B2 and C1 were purchased from Extrasynthese (France).

2. Experimental conditions

The study was carried out during the 2010 season in a 7-year-old commercial vineyard in Naoussa, Northern Greece (40°39'32''N 22°4'21''E, 250 m a.s.l.), planted with *Vitis vinifera* L. cv. Xinomavro (clone V6) grafted onto 110R rootstock. The vineyard was located on a clay loam soil (pH 7.8) and rows were east-west oriented. A divided canopy system (Lyre) and a vertical shoot positioned trellis with two different pruning systems (Royat and Guyot) were applied at 2970 (2.30 × 1.45 m), 3970 (2.30 × 1.10 m) and 3970 (2.30 × 1.10 m) plants/ha, respectively. The Lyre trellis was established with two vertical curtains, 45 and 80 cm apart at the lowest and highest point, respectively; the lowest fruiting wire was set at 55 cm from the ground, and foliage wires at 80, 110 and 145 cm. For the Royat and Guyot trellis, the vines were trained to a single curtain, while the fruiting wire was set at 55 cm from the ground and foliage wires at 85, 110 and 135 cm. In the Lyre treatment, vines were pruned to 10 spurs of 2 buds per vine. The Royat system was composed of a double spur-pruned permanent cordon with 5 spurs of 2 buds per vine, while for the Guyot system, a single cane of 10 buds and one spur of 2 buds were retained. The three treatments were replicated 3 times in a randomized block design, with 20 vines per replication. Canopy management was similarly practiced throughout the growing season and included shoot tucking and positioning and shoot topping to about 30 cm over the top wire, once after fruit set. No leaf removal was performed in any of the three treatments. All treatments were equally irrigated, receiving 42 L per vine during the season.

Vine vigour was assessed by a nondestructive estimation of leaf area per vine at harvest according to the method of Lopes and Pinto (2000). Harvest was conducted simultaneously for the three treatments on 28/08/2010 according to technological maturity surveys (must °brix), and yield per vine was estimated. Samples of 500 berries were collected from each replicate and kept frozen at -20ºC until analysis.

3. Phenolic content of whole berries

50 berries from each replicate were homogenized using Ultra Turrax T25 at 24,000 rpm for 1 min. Total phenol and anthocyanin content was measured according to Iland *et al.* (2004). Briefly, 1 g of the homogenate (in triplicate) was transferred into a centrifuge tube and mixed with 10 mL 50% v/v aqueous ethanol (pH 2) for 1 h. After centrifugation at 3500 rpm for 10 min, 0.5 mL of the supernatant was added to 10 mL 1M HCl and mixed thoroughly for 3 h, then absorbance at 520 nm and 280 nm was recorded.

Total and extractable anthocyanins in the juice were determined as described by Ribéreau-Gayon *et al.* (1999), with slight modifications. An amount of 20 g of the homogenate was macerated for 4 h with two different buffers (pH 1 and pH 3.6). Then, the macerated samples were centrifuged (4000 rpm, 10 min) and the anthocyanin and total phenolic content was measured on the supernatant.

4. Extraction of phenolic compounds from grape seeds and skins

Seeds and skins of 150 berries per replicate were removed manually from grapes, freeze-dried and finally ground to powder. The extraction of skin and seed tannins was carried out according to previously reported methods (Chira *et al.*, 2011; Lorrain *et al.*, 2011). Briefly, 3 g of the obtained powder was first extracted with 25 mL of acetone/water (80:20, v/v) for 3 h and then with 25 mL of methanol/water (60:40, v/v) for 2.5 h. The supernatants were combined and evaporated under reduced pressure at 30ºC to remove organic solvents; the residue was dissolved in water and lyophilized to obtain a crude tannin extract.

4.1 Grape total phenolics, tannin content and antioxidant activity

Part of the crude extract was re-dissolved in a model solution (12% ethanol; 5 g/L tartaric acid; pH 3.5 adjusted with 1N NaOH) for the determination of total phenol content (TPC) by the Folin-Ciocalteu method (Waterman and Mole, 1994), antioxidant activity (Brand-Williams *et al.*, 1995), and chemical astringency estimation through the protein precipitation assay using BSA (Harbertson *et al.*, 2003). Absorbance measurements were recorded on a Jasco V-530 UV/VIS spectrophotometer.

4.2 Preparation and analysis of skins and seeds

The remaining crude extract of seeds and skins was dissolved in a 5% aqueous ethanol solution and extracted thrice with chloroform to remove the lipophilic material (Chira *et al.*, 2011; Lorrain *et al.*, 2011). Then the aqueous phase was extracted thrice with ethyl acetate. Organic and aqueous fractions were collected separately; the organic fraction was concentrated under reduced pressure at 30ºC and lyophilized to obtain a dry powder. The organic fraction contained monomeric and oligomeric proanthocyanidins, while the aqueous fraction contained polymeric tannins (Chira *et al.*, 2011; Lorrain *et al.*, 2011).

The organic fraction of seeds and skins was analyzed according to Kallithraka *et al.* (2006) for the determination of (+)-catechin (C), (-)-epicatechin (EC), epicatechin gallate (ECG), epigallocatechin gallate (EGCG), gallic acid (GA) and procyanidins B1, B2 and C1. The equipment used for HPLC analysis consisted of a Jasco AS-1555 intelligent sampler, a Jasco PU 2089 Plus quaternary gradient pump, a Jasco MD-910 multiwavelength detector and a Jasco LC-Net II / ADC. The column was a Waters Nova-Pak C18 (150 x 3.9 mm, 4 µm). Identification was based on comparing the retention times of the peaks detected with those of original compounds, and on UV/VIS spectral data. Quantification was performed by establishing calibration curves for each compound determined, using the standards. Results were expressed as mg per g dry weight. All analyses were performed in triplicate.

Tannin mDP and %G were determined in both organic and aqueous phases of seed and skin extracts. Tannin extracts were re-dissolved in methanol and were left to react with phloroglucinol solution (50 g/L phloroglucinol, 10 g/L ascorbic acid, 0.1N HCl, in methanol) for 20 min at 50ºC. The reaction was ended by addition of aqueous sodium acetate (40 mM). LC/MS and HPLC analysis were performed for the identification and quantification of phloroglucinol adducts and terminal units (Kennedy and Jones, 2001; Chira *et al.*, 2009; Lorrain *et al.*, 2011). Reaction products were analyzed by LC/MS on a Shimadzu LC/MS 2010A coupled to a single quadrupole mass spectrometer equipped with an electrospray ion source. The mass spectrometer was operated in positive-ion mode. The source's temperature was set at 70ºC, the capillary voltage at 3.5 kV and the cone voltage at -30 eV. The absorbance was recorded at 280 nm and mass spectra were recorded in the range of 50–1500 amu. Separation was performed on a reversed-phase Waters XTerra RR C18 (100 x 4.6 mm, 3.5 µm) column at a flow rate of 0.5 mL/min, using a 20-µL injection volume and the following elution program: eluent A from 80% to 40% in 20 min, which was kept isocratic for further 10 min and then from 40% to 80% in 2 min. Eluent A was 0.1% aqueous acetic acid and eluent B methanol. All analyses were performed in triplicate. The following extension proanthocyanidin units were identified: (-)-epigallocatechin–phloroglucinol, EGCe-P; (+)-catechin-phloroglucinol, Ce-P; (-)-epicatechin-phloroglucinol, ECe-P; and (-)-epicatechin gallate-phloroglucinol, ECGe. (+)-Catechin, Ct; (-)-epicatechin, ECt; and (-)-epicatechin gallate, ECGt were present as terminal tannin units.

5. Analysis of anthocyanin extracts

Anthocyanins were extracted with acidified methanol (0.1% HCl 12 N) from 1 g of dried skin powder at three successive times (4, 18 and 24 h). After centrifugation, the supernatants were combined and analyzed for total anthocyanins (Ribéreau-Gayon *et al.*, 1999) and antioxidant activity (Brand-Williams *et al.*, 1995).

HPLC analysis was carried out for the determination of monomeric anthocyanins on a Restek pinnacle II C18 (250 x 4.6 mm, 5 µm) column at a flow rate of 1 mL/min, using a 10-µL injection volume, detection at 520 nm, and the following elution program: 90% eluent A for 1 min, then from 90% to 50% in 22 min and from 50% to 5% in 10 min, which was kept isocratic for further 2 min. Eluent A was 10% aqueous formic acid and eluent B methanol. Identification was based on comparing retention times and UV spectra of the peaks detected with those of original compounds. Malvidin-3-*O*-acetylmonoglucoside (MlvAc) and malvidin-3-(6-*O*-p-coumaroyl) monoglucoside (MlvCoum) were tentatively identified based on previous observations (Arnous *et al.*, 2002; Kallithraka *et al.*, 2005).

Results were expressed as mg Mlv per g dry skin weight. All analyses were performed in triplicate.

6. Vinification and wine analysis

About 20 kg of the total harvested grapes per vineyard cell were vinified separately. For the winemaking trials, grapes of each replicate were crushed, destemmed and supplemented with 50 mg/L SO_2 (as potassium metabisulfite). Pectolytic enzymes (Safizym Colour, Fermentis, France) at 3 g/hL as well as lyophilized yeasts of the commercial strain SC 22 (Fermentis, France) at 20 g/hL, previously hydrated in water (15 min, 38ºC), were added. Beginning on the second day of fermentation, and for the following days, two punching downs per day were conducted to extract phenolic compounds. After 5 days of maceration, the wines were drained and transferred to other tanks and spontaneous malolactic fermentation was completed after approximately 3 weeks. The wines were racked, supplemented with 50 mg/L SO_2 (as potassium metabisulfite), filtered and bottled until analysis.

Several classical analytical parameters (%vol, hue, colour intensity, total polyphenols - OD280, pH, total acidity) were determined after bottling according to the OIV methods (1990). In addition, their TPC by Folin-Ciocalteu (Waterman and Mole, 1994), total anthocyanin content, ionization index, total tannins (Ribéreau-Gayon *et al.*, 1999), antioxidant activity (Brand-Williams *et al.*, 1995) and monomeric anthocyanins by HPLC (Kallithraka *et al.*, 2005, 2006) were also determined. All analyses were performed in triplicate.

7. Statistics

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 ($p < 0.05$).

RESULTS AND DISCUSSION

1. Vine growth and yield components

The Lyre system significantly increased all vegetative growth components, suggesting that the increased number of retained buds resulted in a higher number of shoots and increased leaf area at the end of canopy growth, compared to the other systems, whether expressed on a per vine, per meter of row or per ha basis (Table 1). Canopy division has been reported to increase canopy volume and surface area compared to non-divided VSP systems in previous studies (Smart, 1985). There was no

Table 1. Growth and yield components of Xinomavro vines measured at harvest

Component	Lyre	Guyot	Royat
Shoots / bud	0.94 ab	0.84 b	1.06 a
Shoots / vine	18.7 a	10.1 b	10.6 b
Shoots / m of row	12.9 a	9.2 b	9.6 b
Shoots / ha	55658 a	39899 b	41884 b
Leaf Area (m^2) / vine	8.80 a	4.80 b	4.90 b
Leaf Area (m^2) / m of row	6.07 a	4.36 b	4.53 b
Leaf Area (m^2) / ha	26136 a	19056 b	19453 b
Clusters / bud	1.06 b	1.36 ab	1.58 a
Clusters / vine	21.1 a	16.33 b	15.78 b
Clusters / m of row	14.6	14.8	14.3
Clusters / ha	62667	64830	62646
Cluster weight (g)	217.1 a	168.4 b	188.2 ab
Yield (kg) / vine	4.58 a	2.75 b	2.97 b
Yield (kg) / m of row	3.15 a	2.50 b	2.70 b
Yield (kg) / ha	13603 a	10918 b	11791 b
Leaf Area to Yield ratio (m^2 / kg)	1.93 a	1.75 ab	1.64 b
Berry weight (g)	1.62	1.58	1.68
Skin weight (g) / berry	0.161	0.172	0.167
Seed weight (g) / berry	0.041	0.042	0.047

Values with different letters within a row are significantly different (Tukey's test, $p < 0.05$).

difference in vigour components between Royat- and Guyot-trained vines.

Bud fruitfulness (clusters per bud) was higher in Royat and lowest in Lyre vines (Table 1). Larger systems (i.e. Lyre) usually tend to induce higher fruitfulness (i.e. higher number of bunches per shoot) due to improved microclimate in the fruiting zone (Swanepoel *et al.*, 1990). Nevertheless, fruitfulness also depends on the balance between shoot and fruit growth components, primarily controlled at winter pruning by retaining a fixed number of nodes according to the vines' vigour potential. The lower fruitfulness of the Lyre system compared to Royat in our experiment may be related to the higher (i.e. double) number of buds left at pruning in the Lyre system, which could have affected source-sink relationships and, thus, flower initiation in developing latent buds. Decline in fruitfulness in response to increased node number has also been reported by Archer and Fouché (1987).

Lyre vines exhibited higher yield per vine, per meter of row and per ha than the Royat and Guyot vines (Reynolds *et al.*, 2004) (Table 1). Changes in yield can be driven by a combination of changes in the number of clusters per vine and cluster weight. In the conditions of our study, the number of clusters per meter of row and per ha was not affected by training system, while cluster weight was higher in the Lyre system. It is therefore possible that the higher weight of the Lyre clusters could be mostly responsible for the higher yield observed in the Lyre system compared to the other treatments. However, our one-year data are not sufficient to draw a more robust conclusion on the effects of training system on yield components and their relative contribution to yield variations.

Berry component weights were similar among the three treatments (Table 1). Berry weights ranged from 1.58 g to 1.68 g, while the skins and seeds represented 10.26% and 2.66% of total grape weight, respectively. This finding suggests that the higher cluster weight in the Lyre system was probably the result of a higher number of berries per cluster. Previous studies also report that changes in cluster weight are mostly a response to changes in berry number rather than in berry weight (Bennett *et al.*, 2005).

Despite of the increased average yield for Lyre, crop loads (i.e. leaf area to yield ratio) were highest in Lyre vines by 18% and 10% compared to Royat and Guyot vines, respectively (Table 1). It has been established that a range of 0.7-1.4 m^2 of exposed leaf area per kg of fruit weight is necessary for full ripening (Howell, 2001), with higher values required for late ripening varieties such as Xinomavro (van Leeuwen *et al.*, 2008). In our conditions, even

Table 2. Phenolic composition of whole berries and skin and seed extracts of Xinomavro berries at harvest

	Lyre	Guyot	Royat
Berries			
anthocyanins (mg / berry)	0.883 a	0.626 b	0.647 b
total anthocyanins (mg / L juice)	252.75 a	201.01 b	217.99 b
extractable anthocyanins (mg / L juice)	143.70 a	121.85 b	123.69 b
anthocyanin extractability (%AE)	43.09	39.46	43.30
total phenolics (au / berry)	2.170 a	1.510 c	1.714 b
Skins *(tannin extract)*			
total phenols (mg gallic acid / g dw)	77.50	66.70	69.75
antioxidant activity (mmol trolox /g dw)	0.134 a	0.119 b	0.118 b
astringency (mg catechin /g dw)	95.19	87.05	95.50
Seeds *(tannin extract)*			
total phenols (mg gallic acid / g dw)	130.05	122.86	121.46
antioxidant activity (mmol trolox /g dw)	0.234	0.213	0.185
astringency (mg catechin /g dw)	78.19 a	69.13 b	65.22 b

Values with different letters within a row are significantly different (Tukey's test, $p < 0.05$). au, absorbance units; dw, dry weight.

though only total leaf area was measured, the divided canopy of the Lyre system would be expected to have a higher proportion of exposed leaves than the other two systems (Reynolds and Vanden Heuvel, 2009) therefore canopy surface area to yield ratio would be further improved as compared to VSP systems.

2. Anthocyanin composition of grape skins

Grapes derived from the Lyre training system contained significantly higher amounts of total and extractable anthocyanins, compared to the other treatments (Table 2). However, no significant differences were recorded for anthocyanin extractability values (%AE), which are an index of grape phenolic maturity in red grapes (Saint-Cricq de Gaulejac *et al.*, 1998). Significant differences were also found regarding the levels of individual anthocyanins in skin extracts, with highest values observed in grapes originating from the Lyre vines (10.5 mg/g dw in sum compared to 8.3 mg/g dw for Royat and Guyot) for all anthocyanin-3-*O*-monoglucosides except MlvAc (Table 3).

It has been reported that anthocyanin profile may be affected by grape variety (González-Neves *et al.*, 2004). For the Xinomavro variety, Mlv and MlvCoum contents were found to be the most important anthocyanins in grape skins in all training systems studied. Mlv represented an average content of 67%, 61% and 64% of total anthocyanin-3-*O*-monoglucosides in Lyre, Royat and Guyot, respectively, while MlvCoum represented 32%, 38% and 36%, respectively, with minor contributions from the other anthocyanins (Table 3). These results do not confirm previous studies reporting that vine training and pruning method may modify grape anthocyanin profile (González-Neves *et al.*, 2004).

Table 3. Concentrations of skin and seed phenolic compounds in Xinomavro berries at harvest

	Lyre	Guyot	Royat
Skins (mg/g dw)			
Anthocyanins			
Dp	0.197 a	0.214 a	0.114 b
Cy	0.152 a	0.108 b	0.101 b
Pt	0.521 a	0.476 ab	0.416 b
Pn	0.945 a	0.746 ab	0.543 b
Mlv	7.096 a	5.327 b	5.101 b
MlvAc	0.379 b	0.441 a	0.470 a
MlvCoum	3.406 a	3.002 b	3.201 ab
Flavan-3-ols			
GA	0.031	0.019	0.020
C	0.051 b	0.041 b	0.117 a
EC	0.030 a	0.008 b	0.056 a
B1	0.012	0.042	0.034
B2	0.022 a	0.010 b	0.037 a
C1	0.006	0.004	0.009
ECG	nd	nd	nd
EGCG	nd	nd	nd
Seeds (mg/g dw)			
Flavan-3-ols			
GA	0.16	0.19	0.19
C	6.93 ab	7.29 a	5.30 b
EC	4.56	4.95	4.06
B1	1.18 ab	1.26 a	0.98 b
B2	1.28	1.33	1.21
C1	1.25	1.08	0.99
ECG	0.09 a	0.08 a	0.07 b
EGCG	0.76 b	1.06 a	1.03 a

Values with different letter within a row are significantly different (Tukey's test, $p < 0.05$).
Dp, delphinidin-3-*O*-monoglucoside; Cy, cyanidin-3-*O*-monoglucoside; Pt, petunidin-3-*O*-monoglucoside; Pn, peonidin-3-*O*-monoglucoside; Mlv, malvidin-3-*O*-monoglucoside; MlvAc, malvidin-3-*O*-acetylmonoglucoside; MlvCoum, malvidin-3-(6-*O*-p-coumaroyl) monoglucoside; nd, not detected.

The results of the comparison between training systems showed that Lyre trellis resulted in an improvement of anthocyanin composition of Xinomavro grape skins, compared to the two VSP systems. This effect could be attributed to multiple factors. First, the higher leaf area per fruit weight of the Lyre vines (Table 1) suggests a better source-sink ratio and a more favourable assimilate distribution during ripening, as also reported for Tannat grapes (González-Neves *et al.*, 2004). Moreover, divided canopies (as in the Lyre trellis) achieve a better ratio of canopy surface area to canopy volume, increasing the proportion of exterior to interior leaves, thereby positively affecting vine carbon balance (Gladstone and Dokoozlian, 2003). Furthermore, divided canopy systems are reported to improve the light microclimate during the day due to decreased canopy density, especially in the fruiting zone (Reynolds and Vanden Heuvel, 2009), as compared to VSP systems. The reduction of shading around the clusters in red varieties was demonstrated to enhance anthocyanin synthesis in previous studies (Bergqvist *et al.*, 2001).

3. Polyphenolic composition and antioxidant activity of skins and seeds

As illustrated in Table 2, seed extracts were richer in phenolic compounds and showed higher antioxidant activity than skin extracts in all samples examined. Total phenolics of whole berries were higher in the Lyre grapes (Table 2) as opposed to Royat and Guyot ones. The results agree with a previous study reporting higher levels of total phenolics in Sangiovese grapes trained to a modified Y-shaped open trellis as compared to a traditional VSP system (Palliotti, 2012). However, training system did not significantly affect skin and seed individual phenol content. Training system affected the antioxidant activity of the skins, with higher values for Lyre. Concerning the astringency sensation, seeds from the Lyre system contained significantly higher contents of precipitable with BSA tannins (Table 2).

The concentration of flavan-3-ol monomers (C, EC, ECG, EGCG) and oligomers (B1, B2, C1) in Xinomavro skins and seeds showed significant differences among the training systems (Table 3). In general, grape skins of the Royat grapes were richer in monomeric flavanols (C, EC), while a different trend was recorded for seeds, where highest C values were measured in the Guyot grapes. Regarding skin oligomeric proanthocyanidin (B1 and C1) concentration, no difference was found among the three treatments. Galloylated monomers (ECG, EGCG) were not detected in any skin extract. Proanthocyanidin B2 was the dominant oligomer in

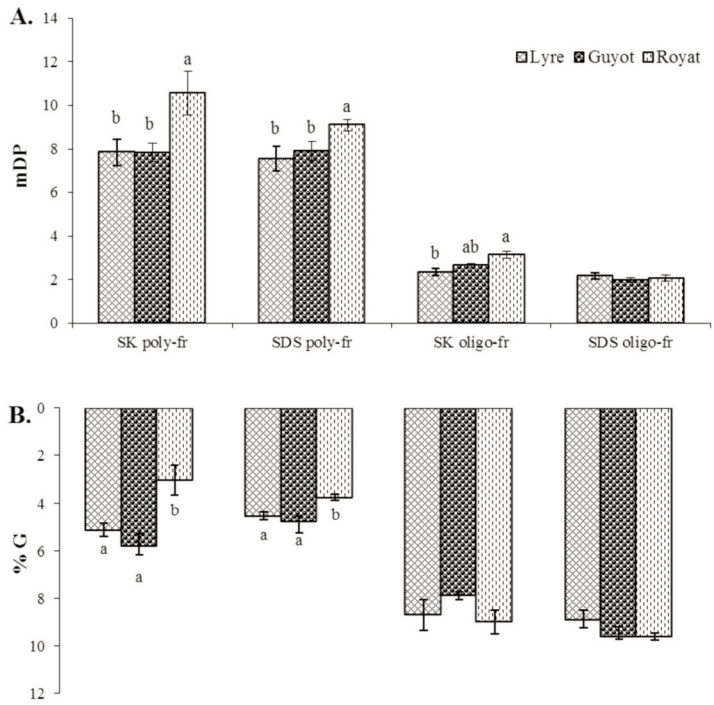

Figure 1. Mean degree of polymerization (mDP, A) and percentage of galloylation (%G; B) of seed oligomeric (SDS oligo-fr) and polymeric fractions (SDS poly-fr) and skin oligomeric (SK oligo-fr) and polymeric fractions (SK poly-fr).

Different letters indicate significant differences among training systems (Tukey's test, $p < 0.05$).

Royat skin extracts, while proanthocyanidin B1 predominated in the skin extracts of the other two systems (Table 3). Regarding seed extracts, Lyre system was characterized by significantly lower concentrations of gallic esters (EGCG), while seeds from the Guyot system were significantly richer ($p < 0.05$) in proanthocyanidin B1. It was also observed that seeds were almost 100 times richer in oligomeric proanthocyanidins as compared to the skins.

4. Structural characterization of skin and seed proanthocyanidins

In all skin samples analyzed, polymers represented 98% and oligomers 2% of the total pool of skin proanthocyanidins, while in seeds, polymers represented 77-81% and oligomers 19-23% of total seed proanthocyanidins. Oligomeric fractions were characterized by a lower mDP but a higher %G than polymeric fractions, with relatively similar trends for seed and skin fractions (Figure 1). This observation

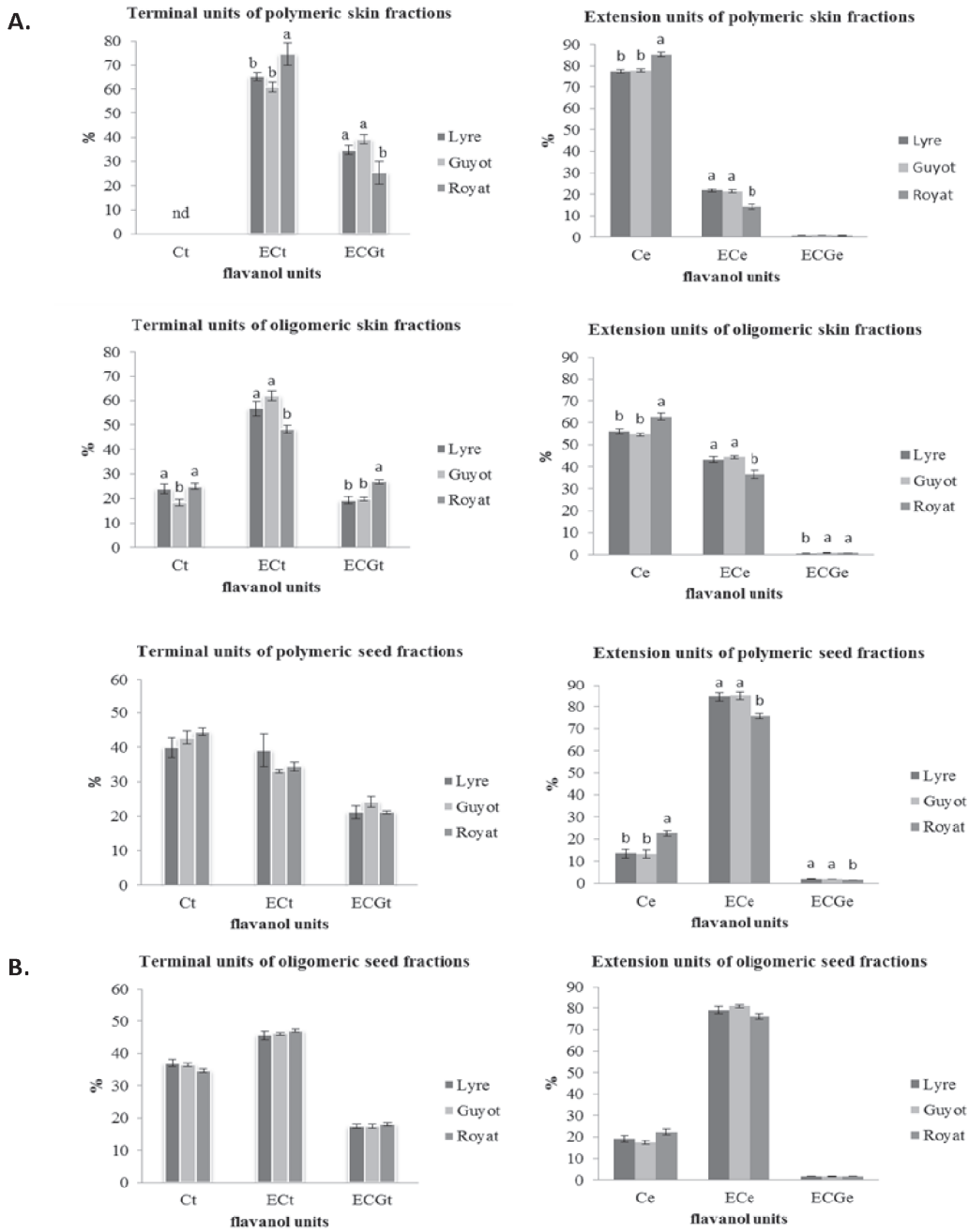

Figure 2. Terminal and extension subunits of oligomeric and polymeric fractions from skins (A) and seeds (B).

Percentage of terminal subunits: %Ct, (+)-catechin; %ECt, (-)-epicatechin; %ECGt, epicatechin gallate; and percentage of extension subunits: %Ce, (+)-catechin; %ECe, (-)-epicatechin; %ECGe, epicatechin gallate. Different letters indicate significant differences among training systems (Tukey's test, $p < 0.05$).

does not confirm previous works in other varieties (Prieur *et al.*, 1994; Sun *et al.*, 2013; Ćurko *et al.*, 2014) reporting that lower mDP values corresponded to low %G.

In general, mDP values of Xinomavro polymeric skin extracts were lower than the respective values reported previously for other varieties (Figure 1A). Data published by several authors (Chira *et al.*, 2009; Bordiga *et al.*, 2011; Lorrain *et al.*, 2011; Ćurko *et al.*, 2014) reported skin mDP values between 16.0 and 35.7 for Merlot, 21.9 and 36.6 for Cabernet Sauvignon, 50.2 for Nebbiolo, 30.0 for Plavacmali, and 40.0 for Babic (Croatian indigenous varieties). Moreover, seed mDP values in both oligomeric and polymeric fractions were lower than those reported for Merlot and Cabernet Sauvignon but close to those reported for Plavacmali and Babic (Ćurko *et al.*, 2014).

Concerning the effect of training system, significant differences were found regarding mDP and %G among the three systems, in both skins and seeds of Xinomavro grapes (Figure 1). Grapes harvested on Royat vines had a higher mDP in the polymeric fraction of both skins and seeds. Regarding oligomeric tannins, Royat showed significantly higher mDP only in berry skins. There were no significant differences in the %G of oligomeric fractions of skins and seeds among the three systems studied. However, in the polymeric fractions, Royat seeds and skins were characterized by the lowest %G compared to the other two treatments.

Figure 2 illustrates the percentage contribution of terminal (t) and extension (e) subunits in polymeric and oligomeric tannin fractions of skins (Figure 2A) and seeds (Figure 2B). In the skins, the main terminal subunit was (-)-epicatechin (%ECt) in both polymer (60-74%) and oligomer fractions (48-61%), while the main extension subunit was (+)-catechin (%Ce). These data are not in agreement with the results obtained by Souquet *et al.* (1996) and Cohen *et al.* (2008), who reported that %Ct was the main terminal and %ECe the main extension unit in Merlot skin tissues. These differences could be due to genotype but might as well be attributed to the different tannin extraction or depolymerization methods employed (phloroglucinolysis vs thiolysis). An interesting finding was the absence of %Ct from the polymeric skin fractions, accompanied by a relatively high (-)-epicatechin gallate (%ECGt) content (24-29%). %ECGe presented the lowest contribution to skin procyanidin extension units.

In the seeds, the dominant terminal subunit of polymeric fraction was Ct (40%), in agreement with the results reported for other varieties (Prieur *et al.*, 1994; Kennedy *et al.*, 2000; Cohen *et al.*, 2008; Bordiga *et al.*, 2011), but ECt was higher in oligomeric seed fractions (45-47%). The main extension subunit in the seeds was ECe in both oligomer (76-80%) and polymer fractions (>75%). (-)-Epicatechin gallate was the compound with the lowest contribution to both terminal (%ECGt) and extension (%ECGe) units. Other authors (Prieur *et al.*, 1994; Kennedy *et al.*, 2000; Cohen *et al.*, 2008; Bordiga *et al.*, 2011) reported similar observations for seed extension subunits of different varieties.

Regarding the effect of training system on the structural properties of proanthocyanidins, Royat was characterized by higher %ECt values in the terminal polymeric skin fractions accompanied by lower %ECGt, while the opposite was observed for oligomeric skin fractions (Figure 2A). In skin extension subunits, both polymeric and oligomeric fractions of the Royat grapes contained higher %Ce and lower %ECe than Lyre and Guyot. In the seeds, no significant differences were found regarding terminal procyanidin subunits of polymeric and oligomeric fractions among the three systems (Figure 2B). Regarding seed extension subunits, polymeric seed fractions of the Royat grapes contained higher %Ce and lower %ECe, similarly to skin proanthocyanidin structure.

Sensory analyses of flavan-3-ol monomers and oligomers have shown that astringency increases with mDP (Clifford, 1986; Robichaud and Noble, 1990). It has also been reported that astringency increases with both %G and molecular weight (Ricardo-da-Silva *et al.*, 1991). Moreover, the structural characteristic of the molecules might also exert a significant influence on their astringency as it has been reported by Kallithraka *et al.* (1997) and Thorngate and Noble (1995) who demonstrated that (-)-epicatechins are significantly more astringent than (+)-catechin when tasted in model wine solutions.

However, it is difficult to relate the differences in astringency and bitterness of grape phenolic extracts to their compositional differences. In the present study, the higher mDP values of Royat seed and skin tannins might result in more astringent wines, but the lower %G values might counteract this effect. Moreover, the lower (-)-epicatechin and the higher (+)-catechin contents of the tannin extension units in the skin and seeds of Royat grapes might also result in a lower astringency sensation potential of the grapes derived from this training system, as

Table 4. Analytical parameters and individual anthocyanins of experimental wines

	Lyre	Guyot	Royat
Analytical parameters			
alcohol (% vol)	15.5	15.5	15.6
pH	3.56 a*	3.41 b	3.42 b
total acidity (g/L)	6.2 b	6.7 a	6.9 a
colour intensity	4.77 a	4.02 b	4.26 b
colour hue	0.68	0.68	0.7
ionization index (%)	66.00 c	87.70 a	71.28 b
total anthocyanins (mg/L)	51.13 a	42.54 b	46.52 b
total tannins (g/L)	2.42	2.04	2.37
OD 280 nm	44	40	45
total phenols (g gallic acid/L)	2.38 a	1.82 b	2.21 a
antioxidant activity (mM trolox)	1.59	1.62	1.56
astringency (mg/L catechin)	238	251	247
Anthocyanins (mg/L)**			
Dp	0.61 a	0.35 b	0.48 b
Cy	nd	nd	nd
Pt	1.48 a	0.99 b	1.04 b
Pn	2.34 a	0.97 b	1.19 b
Mlv	23.25 a	14.38 b	16.05 b
MlvAc	3.80 b	3.43 b	4.59 a
MlvCoum	3.03 a	2.04 b	2.09 b

Values with different letters within a row are significantly different (Tukey's test, $p < 0.05$). Dp, delphinidin-3-*O*-monoglucoside; Cy, cyanidin-3-*O*-monoglucoside; Pt, petunidin-3-*O*-monoglucoside; Pn, peonidin-3-*O*-monoglucoside; Mlv, malvidin-3-*O*-monoglucoside; MlvAc, malvidin-3-*O*-acetylmonoglucoside; MlvCoum, malvidin-3-(6-*O*-p-coumaroyl) monoglucoside; C, (+)-catechin; EC, (-)-epicatechin; ECG, epicatechin gallate; EGCG, epigallocatechin gallate; GA, gallic acid; procyanidins B1, B2 and C1; nd, not detected.

compared to Lyre and Guyot (Kallithraka *et al.*, 1997). The higher astringency of seed tannin extracts in the Lyre berries (Table 2) is supportive of this conclusion.

5. Wine composition

The wines from the different trellis treatments showed similar alcohol contents (Table 4). However, Lyre wines were less acid, as depicted by their lower titratable acidity and higher pH compared to Royat and Guyot. According to a previous study, cordon trained Riesling grapes had lower acidity than divided trellis grapes (Reynolds, 1988). In accordance with skin anthocyanin levels, Lyre wines displayed the highest concentration in total and individual anthocyanins (except malvidin-3-*O*-acetylmonoglucoside) among the three systems (Table 4), thereby leading to a higher colour intensity, despite their lower acidity and ionization index. Previous studies in Cabernet Sauvignon vines trained to Lyre and traditional VSP cordon also showed that wines of the Lyre system were characterized by higher levels of anthocyanins (Katerji *et al.*, 1994). Similarly to whole berry phenolic potential, wines produced from grapes of the Lyre treatment showed higher total phenol contents, although no differences were recorded for total tannins.

No differences were observed in the antioxidant capacity of wines among studied systems. Moreover, differences observed in the structural properties of skin and seed proanthocyanidins among training systems (Figures 1 and 2) were not confirmed by the astringency measured in the experimental wines, which showed similar values.

CONCLUSIONS

Phenolic compounds play a vital role in the quality of red wines. They are responsible for some positive tasting characteristics but also for some unpleasant, negative aspects. Body, fullness and roundness are all organoleptic qualities which characterize premium red wines. On the other hand, bitterness, roughness, harshness, astringency and thinness are faults that

must be avoided. The overall organoleptic impression is based on a harmonious balance between these two types of sensations, directly related to the type and concentration of various phenolic compounds, especially tannins.

Xinomavro grapes are rather poor in anthocyanins and, as a consequence, the resulting wines typically have low colour intensity. On the other hand, Xinomavro grapes are rich in skin and seed tannins, which are characterized by increased astringency and dryness. The results of the present study could be of importance to both vineyard managers and winemakers. By adopting Lyre as a training system, higher anthocyanin levels could be achieved in both grapes and wines. It is also noteworthy that the higher anthocyanin content of Lyre berries was accompanied by a significant gain in yield. On the other hand, Guyot grapes were richer in tannin monomers, while Royat resulted in grapes with relatively higher skin tannins which possibly presented a less pronounced astringent character due to their structural properties (lower %G, lower (-)-epicatechin and higher (+)-catechin contents of the tannin extension units).

With this knowledge, the winemaker could decide on the maceration process parameters (i.e. intensity and length) in order to optimize the quality of Xinomavro wines. In this view, grapes from the Lyre system would be seemingly more suitable for pre-fermentation skin contact in order to extract more anthocyanins, while those of Royat might be more adapted for longer maceration duration to produce full-bodied, balanced wines with longer ageing potential.

Acknowledgments: We would like to express our gratitude to Kir–Yianni Estate (Naoussa, Greece) for managing the experimental vineyard.

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