

## Tannin content and antioxidant capacity of five Greek red grape varieties

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### Summary

Tannins are located in skins and seeds and are responsible for important sensory and quality attributes of red grapes and wines, such as astringency, bitterness and colour stability. However, little is known regarding Greek *Vitis vinifera* varieties. The aim of this study is to evaluate the grape phenolic content and to present data that may contribute to the development of suitable winemaking techniques for these varieties. In this study berry attributes, skin and seed content of tannins and antioxidant capacity from five Greek *Vitis vinifera* varieties, namely 'Mavrotragano', 'Mandilaria', 'Kotsifali', 'Agiorgitiko' and 'Xinomavro' were analyzed.

Significant differences were observed in berry weight and the distribution of berry component mass in mature berries, among the different varieties. 'Mandilaria' and 'Kotsifali' had the heavier berries while the higher contribution of skins and seeds in berry was observed in 'Agiorgitiko' and 'Mavrotragano' grapes respectively. According to the results, the higher content of seed tannins in berries was determined in 'Mandilaria' and the lower in 'Kotsifali'. Finally, varieties with high concentrations of tannins, 'Mandilaria' and 'Mavrotragano', also demonstrated significant high values of antioxidant capacity.

**Key words:** grapes; tannins; BSA method; antioxidant capacity; DPPH; Greek winegrape varieties.

### Introduction

From antiquity to nowadays, viticulture and enology in the Greek area have pointed out a great number of red grape *Vitis vinifera* varieties (KALLITHRAKA *et al.* 2006 and 2014). This continuous and intensive selection of cultivars with genetically distinct enological characteristics and organoleptic properties has favored the production of unique wine styles too (KALLITHRAKA *et al.* 2011, KOUSSISSI *et al.* 2003). Nevertheless, most of these native varieties were becoming rare since they were replaced by international ones due to their well-known grape phenolic and aromatic content (ESTEBAN *et al.* 2001, HARBERTSON *et al.* 2002), which are important tools for the vinification procedures. Recently, there is a great attention into the study of local

varieties from different countries, for example 'Plavac Mali', 'Babic' from Croatia (ĆURKO *et al.* 2014), 'Nebbiolo', 'Nascetta', 'Aglanico' etc. from Italy (BORDIGA *et al.* 2011, RINALDI *et al.* 2014), 'Vranac', 'Kratošija' from Montenegro (PAJOVIĆ-ŠČEPANOVIĆ *et al.* 2016). Although polyphenolic potential and profile of selected Greek grape varieties as 'Agiorgitiko' (KALLITHRAKA *et al.* 2011, KOUNDOURAS *et al.* 2013, PETROPOULOS *et al.* 2017), 'Xinomavro' (KALLITHRAKA *et al.* 2011, KYRALEOU *et al.* 2015), 'Limniona', 'Mavrotragano', etc. (KALLITHRAKA *et al.* 2014) has been carried out in the last years, the survey is incomplete.

It is an indisputable fact that red grape varieties exhibit important amounts of phenolic compounds that originate from berries (HARBERTSON *et al.* 2002, MONAGAS *et al.* 2003) and contribute to the quality and organoleptic character of the wine. Amongst them, tannins and anthocyanins are both of great technological interest since they have a great impact on astringency, bitterness, color stability and ageing potential (KALLITHRAKA *et al.* 1997, PELEG *et al.* 1999, CHIRA *et al.* 2012, CASASSA *et al.* 2013, KYRALEOU *et al.* 2016b).

A great amount of grape tannins is biosynthesized during the first phases of berry growth, reaching its maximum level around veraison (DOWNEY *et al.* 2003). Phenolic content, composition and accumulation in berry seeds and skin can be affected by grape variety (BORDIGA *et al.* 2011), environment (KOUNDOURAS *et al.* 2006) and agricultural practices (BONADA *et al.* 2015, KYRALEOU *et al.* 2017). Seed and skin tannins differ biochemically regarding to their content and structural composition (DOWNEY *et al.* 2003, BORDIGA *et al.* 2011). Generally, skin tannins are presented larger (with a higher mean polymerization degree) and with a proportion of prodelphinidins in their structural composition in contrast to seed tannins (ĆURKO *et al.* 2014; KYRALEOU *et al.* 2017). A number of scientific studies is dedicated to tannin extractability from seeds and skins (CERPA-CALDERÓN and Kennedy 2008, CASASSA *et al.* 2013) during fermentation, grape variety is one of the main factors, but the phenolic ripeness and winemaking techniques are also important as berry treatment (crushed or no crushed), time of skin contact and addition of fining agents (CERPA-CALDERÓN and KENNEDY 2008, HANLIN *et al.* 2011, CASASSA *et al.* 2013). The level of tannins measured in a sample, apart from other parameters, strongly varies with the method employed. Even if molecular methods appear to approach better the absolute amount of total extractable tannins, BSA method is still useful for viticultural management or winemaking purposes given the

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fact that protein precipitation is strongly correlated with perceived astringency (KENNEDY *et al.* 2006).

Especially dimeric, trimeric, oligomeric, or polymeric proanthocyanidins account for most of the superior antioxidant capacity of grape seeds (YILMAZ and TOLEDO 2004). Grape seed extracts are considered to elicit higher antioxidant capacity than skin extracts and the difference in the concentration of monomeric and oligomeric flavan-3-ols, which are both higher in seed extracts (KYRALEOU *et al.* 2017), may have a key-role in this observation (PLUMB *et al.* 1998). Antiradical activity in grapes and wines has been measured with a number of methods and expressed differently depending on the reagent used (KEFALAS *et al.* 2003, ROCKENBACH *et al.* 2011). The DPPH assay used in the present study is related to the capacity of extracts to directly transfer hydrogen to a radical (DPPH) and estimates how capable an antioxidant is in preventing reactive radical species from reaching several components of the biological systems (KATALINIC *et al.* 2010).

The focus of this project is to investigate the berry attributes and phenolic content of five Greek *Vitis vinifera* varieties in order to propose these data as an indispensable tool in choosing the appropriate grape varieties and selecting the vinification technique which will enable the production of high quality wines.

### Material and Methods

**Grape samples:** This study was conducted in five Greek red winegrape varieties *Vitis vinifera* L. 'Mavrotragano' (VVC 40210), 'Mandilaria' (VVC 7300), 'Kotsifali' (VVC 6446), 'Agiorgitiko' (VVC 102) and 'Xinomavro' (VVC 13284). Samples of 500 berries were collected at commercial harvest (Table) from 45 vineyards of 9 different regions, the island of Santorini, the island of Paros, the island of Crete, Naousa, Amyntaio, Goumenissa, Drama, Thessaloniki (North Greece), Nemea and Rapsani (Central Greece). An amount of 100 berries was weighed and the skins and seeds were manually removed to determine mean berry mass and the distribution of berry mass components, % skin per berry and % seed per berry weight ratio, among the different varieties. Analytical grape parameters (°Baumé, pH, titratable acidity) were also determined in a sub-sample of 200 berries.

**Analysis of phenolic compounds and antioxidant capacity:** The skins and seeds from 100 berries were freeze-dried and were ground to obtain powder. Different solvents were used in skin or seed powder to extract tannins, according to a previously published method (KYRALEOU *et al.* 2015), and then the lipophilic material was removed by chloroform. No fractionation was carried out to separate oligomeric and polymeric proanthocyanidins and the final total tannin extracts were dissolved in methanol. The protein precipitable tannins, (PPT) were determined using bovine serum albumin (BSA) (HARBERTSON *et al.* 2002, KYRALEOU *et al.* 2017). Absorbance measurements at 510 nm were recorded on a V-530 UV/VIS spectrophotometer (Jasco, Victoria, BC, Canada) and the concentrations in the methanol solutions were calculated according to HARBERTSON *et al.* (2002). The concentrations in grapes were expressed in mg catechin/g fresh weight (f.w.) of seeds (sd) or skins (sk) and in mg catechin/berry.

A part of the skin powder (1 g) was also used to extract anthocyanins with acidified methanol (1 mL HCl·L<sup>-1</sup>) and then monomeric anthocyanins were determined by high-performance liquid chromatography according to previous method (KALLITHRAKA *et al.* 2005, KYRALEOU *et al.* 2016a) for each sample. Total anthocyanins (TAnth) were calculated as the sum of all identified peaks and the concentration was expressed as mg·g<sup>-1</sup> skin fresh weight of malvidin-3-O-glycoside (Mlv) equivalents.

The antioxidant capacity (AC) was estimated using the 2,2'-diphenyl-1-picrylhydrazyl radical scavenging method and it was expressed as mmol Trolox·g<sup>-1</sup> f.w. of seeds or skins and mmol Trolox·berry<sup>-1</sup>. Absorbance measurements were recorded at 515 nm on a V-530 UV/VIS spectrophotometer (Jasco, Victoria, BC, Canada) and all analyses were performed in triplicate, for every sample.

**Statistical analysis:** Data statistical analysis was submitted to a variance analysis (ANOVA), on Statistica V.7 Software (StatsoftInC., Tulsa, OK, USA). Comparison of mean values was performed using Tukey's HSD test when samples were significantly different after ANOVA ( $p < 0.05$ ).

### Results and Discussion

**Berry features:** The harvest day of each sample varied according to the region and the variety, which influ-

Table

Berry characteristics of grapes measured at harvest

Variety	°Brix	Total acidity	pH	Berry weigh	% seeds/ berry	% skins/ berry	% flesh/berry	skin/flesh
Mavrotragano	24.3 ± 0.2 a*	3.8 ± 0.2 bc	3.7 ± 0.1 a	1.6 ± 0.1 c	6.4 ± 0.5 a	5.4 ± 0.4 d	88.2 ± 1.0 b	0.062 ± 0.01 c
Mandilaria	20.8 ± 0.3 c	4.0 ± 0.3 bc	3.6 ± 0.1 a	2.3 ± 0.2 ab	4.9 ± 0.5 b	6.6 ± 0.5 c	88.5 ± 1.0 b	0.074 ± 0.01 c
Kotsifali	22.1 ± 0.9ab	4.6 ± 0.5 b	3.6 ± 0.1 a	2.4 ± 0.1 a	2.7 ± 0.1 d	6.3 ± 0.3 c	90.9 ± 0.3 a	0.070 ± 0.01 c
Agiorgitiko	22.1 ± 0.2 b	3.5 ± 0.2 c	3.8 ± 0.1 a	2.0 ± 0.1 b	3.8 ± 0.2 c	10.6 ± 0.9 a	85.6 ± 1.1 c	0.124 ± 0.01 a
Xinomavro	22.2 ± 0.3 b	6.1 ± 0.2 a	3.3 ± 0.0 b	1.9 ± 0.1 b	2.7 ± 0.1 d	8.7 ± 0.5 b	88.7 ± 0.6 b	0.098 ± 0.01 b

\* Values are expressed as the means ± standard error. Values followed by different letters indicate significant differences among irrigation treatments (Tukey's test,  $p < 0.05$ ).

enced the maturity of the berries. 'Mavrotragano' grapes reached the higher maturity which was 24.3 °Brix, while 'Mandilaria' had the lowest, 20.8 °Brix. 'Kotsifali', 'Agiorgitiko' and 'Xinomavro' had an average of technological maturity level at 22.1 and 22.2 °Brix (Table). Titratable acidity (TA) values, ranged between 3.5 and 6.1 g tartaric acid·L<sup>-1</sup>. The lower pH and the higher value of titratable acidity (TA) was determined in 'Xinomavro' grapes (pH 3.3 and TA 6.1 g tartaric acid·L<sup>-1</sup>) and is also the main characteristic of this variety, its name comes from the words sour (xino) and black (mavro). The pH level is of great importance for the produced wine because it has been associated with the astringency sensation (KALLITHRAKA *et al.* 1997). The lowest TA was determined in 'Agiorgitiko' grapes (3.5 g tartaric acid·L<sup>-1</sup>), followed by 'Mavrotragano' (3.8 g tartaric acid·L<sup>-1</sup>) and 'Mandilaria' (4 g tartaric acid·L<sup>-1</sup>). Although 'Kotsifali' had the second highest value of TA (4.6 g tartaric acid·L<sup>-1</sup>), no significant differences of pH values were observed among the last four varieties mentioned (Table).

Berry size is an important yield parameter. 'Mavrotragano', had the smallest berries with 1.6 g and 'Kotsifali' with 'Mandilaria' the biggest ones (Table). The distribution of berry components (seeds, skins and flesh) in mature berries was significantly different between the five varieties. As it has been previously reported, these attributes are related to the grape cultivar (BORDIGA *et al.* 2011, SHELLIE, 2011), however the skin thickness can also be sensitive to high temperatures which leads to cell division, thicker skins and increased skin to flesh ratio (PALLIOTTI *et al.* 2011). 'Mavrotragano' had the highest contribution of seeds (6.4 %) in the berry mass, while 'Kotsifali' and 'Xinomavro' had the lowest (2.7 %) (Table). The highest ratio of skin per berry weight was observed in 'Agiorgitiko' (10.6 %), followed by 'Xinomavro' grapes (8.7 %) and the lowest in 'Mavrotragano' (5.4 %). The skin per flesh portion in berries is an important feature for the quality and sensory characteristics of the wine. Skins are rich in phenolic and aromatic compounds which influence the aroma, flavour, and colour attributes of the berries. These compounds extract to the must during vinification and their contribution in organoleptic character of the wine is more significant when the ratio of skins to flesh is increasing. As it appears in the Table, skin/flesh values were higher for 'Agiorgitiko' (0.128) and 'Xinomavro' (0.098) and lower for the other three varieties 'Kotsifali' (0.070), 'Mandilaria' (0.074) and 'Mavrotragano' (0.062). These attributes, % seeds/berry, % skins/berry, % flesh·berry<sup>-1</sup> and skin·flesh<sup>-1</sup>, were not correlated ( $p > 0.01$ ) to the berry weight differences and they could be a varietal characteristic. However, it was observed a high negative correlation ( $r = -0.845$ ,  $p < 0.01$ ) between % skins·berry<sup>-1</sup> and % flesh·berry<sup>-1</sup>. In previous studies, smaller berry size results to higher relative contribution of skins and seeds to total berry mass (KOUNDOURAS *et al.* 2013), but only for the same variety.

**Phenolic composition:** The results of seeds and skin tannins, PPTfw (mg catechin·g<sup>-1</sup> f.w.) as a concentration of fresh weight of seeds or skins and the total values in berries PPTb (mg catechin·berry<sup>-1</sup>) as content per berry and PPTbg (mg catechin·g berry<sup>-1</sup>) as concentration per g·berry<sup>-1</sup> are present in Fig. 1. Although in the four of the five varieties the contribution of seeds in berry mass was

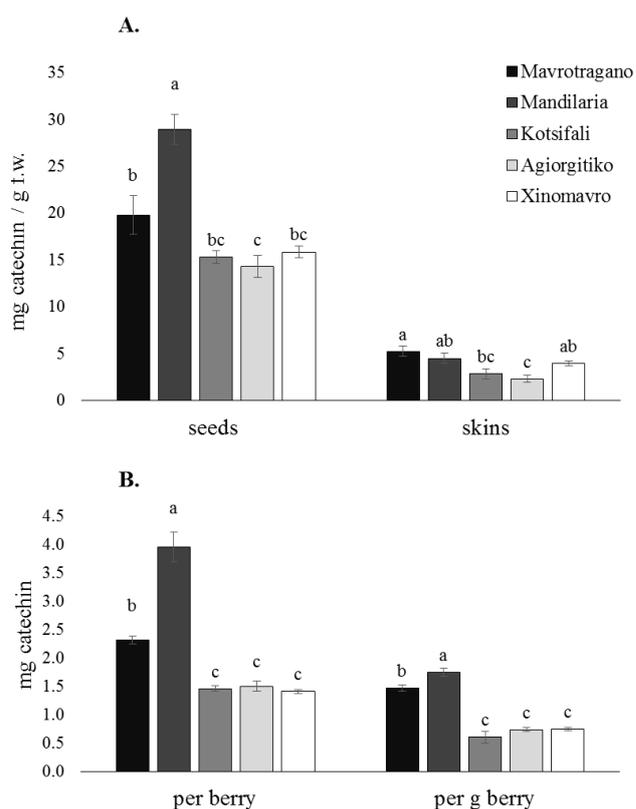


Fig. 1: Protein precipitable tannins (PPT) from the varieties 'Mavrotragano', 'Mandilaria', 'Kotsifali', 'Agiorgitiko' and 'Xinomavro'. A: concentration in mg catechin·g<sup>-1</sup> fresh weight of seeds and skins. B: content of mg catechin per berry and per g berry. Bars indicate ± S.E. of the mean value. Significant differences among treatments are indicated by different letters (Tukey's test,  $p < 0.05$ ).

lower compared to skins (Table), tannin concentrations in seeds were higher compared to skins, for all the varieties as it was previously observed (BONADA *et al.* 2015, KYRALEOU *et al.* 2017). Generally, skin tannins are presented in lower concentrations compared to seed tannins and are easily extracted during fermentation, however the seed tannins can also contribute to the profile of tannins found in the respective wines and give structure to the wine but can also impart excessive astringency (MONAGAS *et al.* 2003).

Significant variations in the levels of PPTfw in seeds and skins were observed among the varieties. The above method determines condensed trimer to octamer tannins (HARBERTSON *et al.* 2014) which contribute to astringency and no low molecular size tannins, monomers and dimers, which are contributing mainly to bitterness (CASASSA *et al.* 2013). Moreover, protein precipitation by BSA was strongly correlated with astringency and it has been suggested as a useful tool for understanding the effect of tannin structure modification on astringency perception (KENNEDY *et al.* 2006).

According to Fig. 2, 'Mandilaria' had the highest PPTfw in seeds (28.9 mg catechin·g<sup>-1</sup> f.w.), with levels higher by 31.6 % from 'Mavrotragano', 47.1 % from 'Kotsifali', 50.6 % from 'Agiorgitiko' and 45.2 % from 'Xinomavro' seed tannins concentration. The high tannin concentration of 'Mandilaria' cannot be associated with the lower maturity level, compared to the other four varieties, since the greater concentration of PPTfw in skins (5.2 mg·g<sup>-1</sup> f.w.) and also

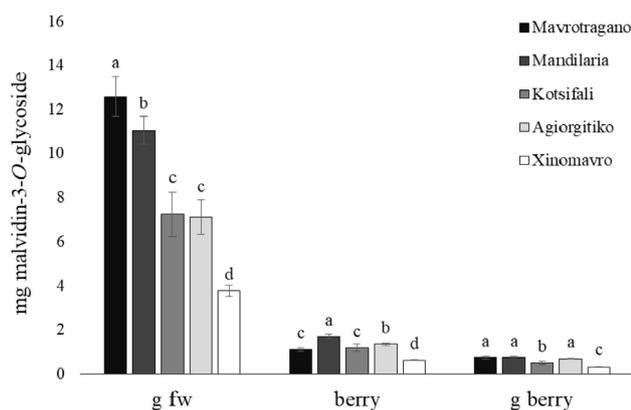


Fig. 2: Total anthocyanins (TAnth) from the varieties 'Mavrotragano', 'Mandilaria', 'Kotsifali', 'Agiorgitiko' and 'Xinomavro'. Concentration in mg  $\text{Mlv} \cdot \text{g}^{-1}$  fresh weight of skins, content of mg  $\text{Mlv}$  per berry and per g berry. Bars indicate  $\pm$  S.E. of the mean value. Significant differences among treatments are indicated by different letters (Tukey's test,  $p < 0.05$ ).

the second highest concentration of seed tannins ( $19.8 \text{ mg} \cdot \text{g}^{-1}$  f.w.) was observed in 'Mavrotragano', which had the most mature grapes (Table). This observation indicates that the content of tannins is primarily varietal dependent and the maturity of grapes effect mostly the extraction of tannins during vinification procedures (CASASSA *et al.* 2013). Study focused on the way of skin and seed tannins behaviour during maceration showed that skin tannins are extracted in higher concentrations and more rapid than seeds (CERPA-CALDERÓN and KENNEDY 2008), however after 10 d of maceration process seed tannins were more extractable especially in crushed berries. Additionally, it was observed that only the 30 % of total skin tannins are extracted during maximum maceration period (CERPA-CALDERÓN and KENNEDY 2008).

Among the varieties, 'Agiorgitiko' showed the lower concentrations of tannins in seeds ( $14.4 \text{ mg} \cdot \text{g}^{-1}$  f.w.) and skins ( $2.2 \text{ mg} \cdot \text{g}^{-1}$  f.w.). Seed tannins can be a varietal characteristic as they change a little from veraison to harvest, on the contrast of skin tannins which can decrease during berry ripening (KYRALEOU *et al.* 2017) due to their ability to associate with tannins and cellular components, such as lignins, proteins and cell wall polysaccharides (DOWNEY *et al.* 2003) and also texture changing of skin during ripening, which is making tannins less extractable (BORDIGA *et al.* 2011). In previous studies phenolic accumulation and content can alter under multiple factors including environmental conditions (KOUNDOURAS *et al.* 2006) or viticultural practices (KYRALEOU *et al.* 2015 and 2017), however there is evidence that the response to viticultural practices, as water supply, is mainly dependent on the variety (NICULCEA *et al.* 2015).

Total tannins were also expressed as content (PPTb) and concentration (PPTbg) in berry fresh mass, an additional indicator that these differences were due to grape variety and in some cases were related to berry size. 'Mandilaria' was the richest variety, with a PPTb content of  $3.9 \text{ mg catechin} \cdot \text{berry}^{-1}$  and a level higher with 41.6 %, 63.2 %, 62.1 % and 64.5 % of 'Mavrotragano', 'Kotsifali', 'Agiorgitiko' and 'Xinomavro', respectively (Fig. 1). A lower content of PPTb was observed in 'Xinomavro' berries with  $1.4 \text{ mg}$

$\text{catechin} \cdot \text{berry}^{-1}$ . When tannins were expressed as concentration of mg catechin per g berry the PPTbg of 'Mandilaria' was estimated at  $1.7 \text{ mg catechin per g berry}$  which was higher by 15.8 %, 65.5 %, 57.8 % and 57.4 % compared to the other varieties 'Mavrotragano', 'Kotsifali', 'Agiorgitiko' and 'Xinomavro', respectively. No significant differences in PPTb and PPTbg were observed among 'Kotsifali', 'Agiorgitiko' and 'Xinomavro'. These results are in agreement to previous findings that wines from 'Mandilaria' appeared to be more astringent compared to those produced from 'Agiorgitiko' or 'Xinomavro' grapes (KALLITHRAKA *et al.* 2011). Moreover, the differences among content and concentration values indicate the importance of berry size at harvest and the great impact of this parameter on the decisions of the vinification process that an oenologist must follow.

Total anthocyanins are presented in Fig. 2. They were determined in skins and expressed as TAnth in mg  $\text{Mlv} \cdot \text{g}^{-1}$  fresh weight (f.w.) of skins, mg  $\text{Mlv}$  per berry, the content per berry and mg  $\text{Mlv}$  per g berry, the concentration per g berry. TAnth in skins fresh weight ranged from  $3.7$ - $12.6 \text{ mg Mlv per g f.w.}$ , according to the variety. 'Xinomavro' had the lowest concentration and 'Mavrotragano' the highest (Fig. 2). However, when the results expressed in berry content 'Mandilaria' had the highest  $\text{Mlv}$  values,  $1.6 \text{ mg} \cdot \text{berry}^{-1}$ , 35 % higher than 'Mavrotragano', which was as a consequence of the small berry size of this variety (Table). TAnth from 'Agiorgitiko' and 'Kotsifali' grapes did not differ significantly when expressed in fresh weight of skins, but 'Kotsifali' had lower content in berries than 'Agiorgitiko'. It was observed that concentrations in  $\text{g} \cdot \text{berry}^{-1}$  in 'Mavrotragano', 'Mandilaria' and 'Agiorgitiko' had no significant differences. 'Xinomavro' had the lowest values compared to the other varieties in all expressions. Previous studies had also indicated that 'Xinomavro' is poor in anthocyanins compared to 'Mandilaria' (KALLITHRAKA *et al.* 2005, KALLITHRAKA *et al.* 2009) and 'Agiorgitiko' grapes (KALLITHRAKA *et al.* 2009).

It has been stated that wines produced from 'Agiorgitiko' grapes had a smooth mouth-feel and lower astringency, whereas wines from 'Xinomavro' grapes were rated more astringent (KALLITHRAKA *et al.* 2011, KOUSSISSI *et al.* 2003). In the present study 'Agiorgitiko' had a lower concentrations of seed and skin tannins compared to 'Xinomavro' grapes, while there were no significant differences between their concentrations per g berry. As it was aforementioned, 'Xinomavro' grapes had higher acidity and lower content of anthocyanins which are attributes that influence the vinification procedures, mainly the maceration time which affects the extraction of all phenolic compounds, and contribute to the organoleptic character of a wine. It has been stated that astringency increases significantly with the decrease of wine pH (KALLITHRAKA *et al.* 1997) in addition, the presence of anthocyanins, through reactions between anthocyanin and flavanols, might delay the flavanol-protein interactions which can affect the astringent sensation (KALLITHRAKA *et al.* 2011).

**Antioxidant capacity of seeds and skins:** The antioxidant capacity (AC) of grape seeds and skins ranged from  $0.14$  to  $0.24 \text{ mmol Trolox} \cdot \text{g}^{-1}$  f.w. and  $0.04$  to  $0.08 \text{ mmol Trolox} \cdot \text{g}^{-1}$  f.w., respectively (Fig. 3).

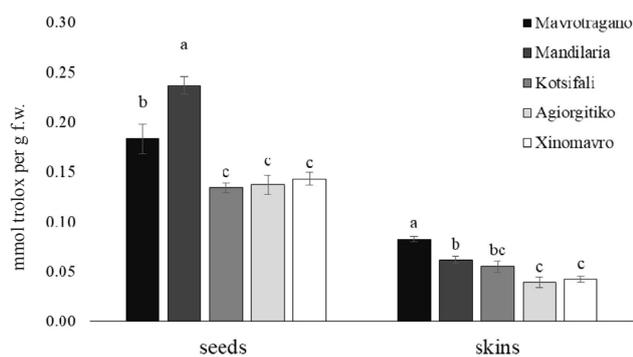


Fig. 3: Antioxidant capacity (AC) of tannin extracts from the varieties 'Mavrotragano', 'Mandilaria', 'Kotsifali', 'Agiorgitiko' and 'Xinomavro'. Concentration in mmol Trolox·g<sup>-1</sup> fresh weight of seeds and skins. Bars indicate ± S.E. of the mean value. Significant differences among treatments are indicated by different letters (Tukey's test,  $p < 0.05$ ).

Both seeds and skins from grapes are good sources of tannins with significant antioxidant capacity. Seeds showed higher AC compared to skins, which could be associated with the greater tannin concentrations and the different structure of condensed tannins in seeds and skins (KYRALEOU *et al.* 2017). According to previous studies, the presence of galloylated subunits, usually determined in seed extracts (KYRALEOU *et al.* 2017), seems to confer antioxidant capacity to a mixture (PLUMB *et al.* 1998). The highest AC was determined in 'Mandilaria' for the seed tannin extracts (0.24 mmol Trolox·g<sup>-1</sup> f.w) and in 'Mavrotragano' for the skin tannin extracts (0.08 mmol Trolox·g<sup>-1</sup> f.w.). It was previously reported that grapes with darker skin had higher AC than those with lighter skin (YILMAZ and TOLEDO 2004), as it was also observed in the current study. These concentrations are higher compared to those stated in previous reports which may be due to the different variety or extraction method (YILMAZ *et al.* 2015). Positive correlations between PPT concentration and AC were observed for the seeds ( $r = 0.831$ ,  $p < 0.01$ ) and the skins ( $r = 0.727$ ,  $p < 0.01$ ) and are presented in Fig. 4. Previous studies have also reported high correlation between phenolic concentration and antioxidant capacity in seeds, skins, pomaces and wines with various antiradical methods (LINGUA *et al.* 2016, WEIDNER *et al.* 2012, YILMAZ *et al.* 2015, ROCKENBACH *et al.* 2011).

### Conclusions

In this study the phenolic composition of five Greek *Vitis vinifera* varieties was evaluated. The results indicated that Greek varieties are particularly rich in tannins and anthocyanins. The seeds of 'Mandilaria' and the skins of 'Mavrotragano' were the richer in total tannin concentration, while 'Agiorgitiko' was the variety with lower tannin content in both seeds and skins. The concentration of tannins was not correlated with the maturation (sugar content) of berries. 'Xinomavro' was characterized by the lowest anthocyanic concentration while 'Mavrotragano' was the richest; however, when the results were expressed as mg per berry 'Mandilaria' was richer than 'Mavrotragano' due to its small

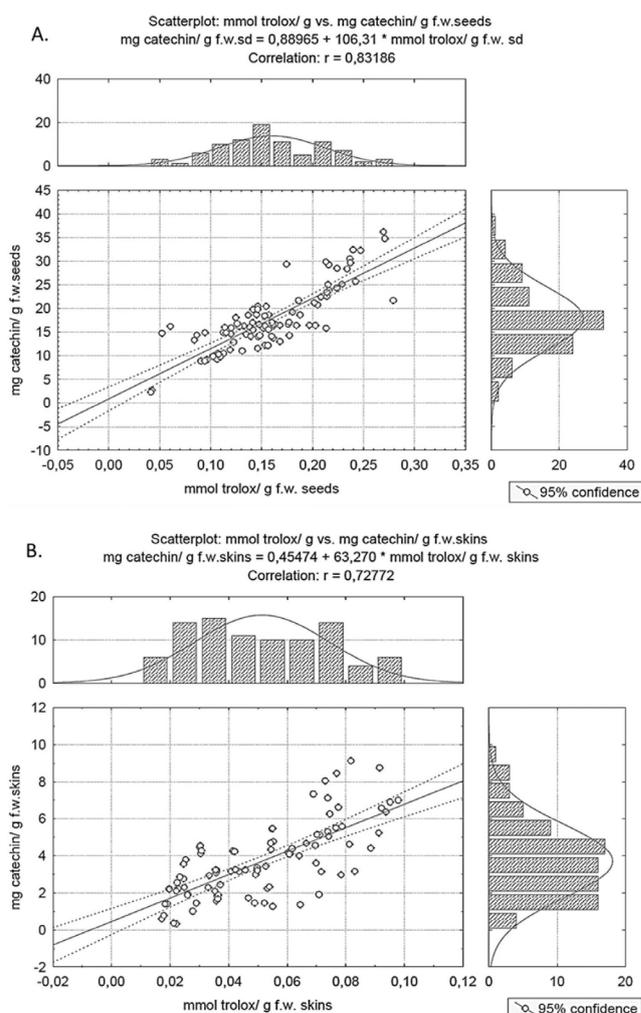


Fig. 4: Correlation between antioxidant capacity. **A:** PPT in seed tannin extracts. **B:** PPT in skin tannin extracts.

berry size. 'Xinomavro' was the variety with the lowest anthocyanin content expressed either as mg·fresh weight<sup>-1</sup> or mg·berry<sup>-1</sup>. Antioxidant capacity values of grape skins and seeds were particularly high, in comparison with relative values reported in literature, and were correlated with their tannin concentrations. According to the results, total acidity was not associated to maturity level and it was mainly a varietal characteristic. In addition, skin, seed and flesh weight contribution to the total weight of berries was not correlated to berry weight and these measured parameters could be a varietal characteristic, too, however a negative correlation between the ratio of % skins·berry<sup>-1</sup> and % flesh·berry<sup>-1</sup> was observed. The results presented, is a first attempt to elucidate the phenolic composition of the indigenous grape varieties since this parameter is of high importance for the wine industry for which the optimization of wine sensory properties remains a priority. These data could be used as a tool in selecting the most suitable grape varieties for the production of specific wine types and the technological applications, such as maceration and maturation time, for the production of high quality wines. However, further research is needed to better understand the varietal differences of grapes such as the determination of their volatile composition and the organoleptic properties of the corresponding wines.

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## References

- BONADA, M.; JEFFERY, D.; PETRIE, P.; MORAN, M.; SADRAS, V.; 2015: Impact of elevated temperature and water deficit on the chemical and sensory profiles of Barossa Shiraz grapes and wines. *Aust. J. Grape Wine Res.* **21**, 240-253.
- BORDIGA, M.; TRAVAGLIA, F.; LOCATELLI, M.; COISSON, J.; ARLORIO, M.; 2011: Characterization of polymeric skin and seed proanthocyanidins during ripening in six *Vitis vinifera* L. cv. *Food Chem.* **127**, 180-187.
- CASASSA, L. F.; BEAVER, C.; MIRELES, M.; HARBERTSON, J. F.; 2013: Effect of extended maceration and ethanol concentration on the extraction and evolution of phenolics, colour components and sensory attributes of Merlot wines. *Aust. J. Grape Wine Res.* **19**, 25-39.
- CERPA-CALDERÓN, F. K.; KENNEDY, J.; 2008: Berry integrity and extraction of skin and seed proanthocyanidins during red wine fermentation. *J. Agric. Food Chem.* **56**, 9006-9014.
- CHIRA, K.; JOURDES, M.; TEISSEDE, P. L.; 2012: Cabernet Sauvignon red wine astringency quality control by tannin characterization and polymerization during storage. *Eur. Food Res. Technol.* **234**, 253-261.
- ĆURKO, N.; KOVAČEVIĆ GANIĆ, K.; GRACIN, L.; ĐAPIĆ, M.; JOURDE, M.; TEISSEDE, P.; 2014: Characterization of seed and skin polyphenolic extracts of two red grape cultivars grown in Croatia and their sensory perception in a wine model medium. *Food Chem.* **145**, 15-22.
- DOWNNEY, M. O.; HARVEY, J. S.; ROBINSON, S. P.; 2003: Analysis of tannins in seeds and skins of Shiraz grapes throughout berry development. *Aust. J. Grape Wine Res.* **9**, 15-27.
- ESTEBAN, M.; VILLANUEVA, M.; LISARRAGUE, J.; 2001: Effect of irrigation on changes in the anthocyanin composition of the skin cv. Tempranillo (*Vitis vinifera* L.) grape berries during ripening. *J. Sci. Food Agric.* **81**, 409-420.
- GUENDEZ, R.; KALLITHRAKA, S.; MAKRIS, D. P.; KEFALAS, P.; 2005: Determination of low molecular weight polyphenolic constituents in grape (*Vitis vinifera* sp.) seed extracts: Correlation with antiradical activity. *Food Chem.* **89**, 1-9.
- HANLIN, R. L.; KELM, M.; WILKINSON, K.; DOWNEY, M.; 2011: Detailed characterization of proanthocyanidins in skin, seeds, and wine of Shiraz and Cabernet Sauvignon wine grapes (*Vitis vinifera*). *J. Agric. Food Chem.* **59**, 13265-13276.
- HARBERTSON, J. F.; KILMISTER, R. L.; KELM, M. A.; DOWNEY, M. O.; 2014: Impact of condensed tannin size as individual and mixed polymers on bovine serum albumin precipitation. *Food Chem.* **160**, 16-21.
- HARBERTSON, J.; KENNEDY, J.; ADAMS, D.; 2002: Tannin in skins and seeds of Cabernet Sauvignon, Syrah, and Pinot noir berries during ripening. *Am. J. Enol. Vitic.* **53**, 54-59.
- KALLITHRAKA, S.; ALIAJ, L.; MAKRIS, D. P.; KEFALAS, P.; 2009: Anthocyanin profiles of major red grape (*Vitis vinifera* L.) varieties cultivated in Greece and their relationship with *in vitro* antioxidant characteristics. *Int. J. Food Sci. Technol.* **44**, 2385-2393.
- KALLITHRAKA, S.; BAKKER, J.; CLIFORD, M.; 1997: Effect of pH on astringency in model solutions and wines. *J. Agric. Food Chem.* **45**, 2211-2216.
- KALLITHRAKA, S.; KIM, D.; TSAKIRIS, A.; PARASKEVOPOULOS, I.; SOLEAS, G.; 2011: Sensory assessment and chemical measurement of astringency of Greek wines: Correlations with analytical polyphenolic composition. *Food Chem.* **126**, 1953-1958.
- KALLITHRAKA, S.; KOTSERIDIS, Y.; KYRALEOU, M.; PROXENIA, N.; TSAKIRIS, A.; KARAPETROU, G.; 2014: Analytical phenolic composition and sensory assessment of selected rare Greek cultivars after extended bottle ageing. *J. Sci. Food Agric.* **95**, 1638-1647.
- KALLITHRAKA, S.; MOHDALYA, A.; MAKRIS, D. P.; KEFALAS, P.; 2005: Determination of major anthocyanin pigments in Hellenic native grape varieties (*Vitis vinifera* sp.): association with antiradical activity. *J. Food Comp. Anal.* **18**, 375-386.
- KALLITHRAKA, S.; TSOUTSOURAS, E.; TZOUROU, E.; LANARIDIS, P.; 2006: Principal phenolic compounds in Greek red wines. *Food Chem.* **99**, 784-793.
- KATALINIC, V.; MOZINA, S. S.; SKROZA, D.; GENERALIC, I.; ABRAMOVIC, H.; MILOS, M.; BOBAN, M.; 2010: Polyphenolic profile, antioxidant properties and antimicrobial activity of grape skin extracts of 14 *Vitis vinifera* varieties grown in Dalmatia (Croatia). *Food Chem.* **119**, 715-723.
- KEFALAS, P.; KALLITHRAKA, S.; PAREJO, I.; MAKRIS, D.; 2003: Note: a comparative study on the *in vitro* antiradical activity and hydroxyl free radical scavenging activity in aged red wines. *Food Sci. Technol. Int.* **9**, 383-387.
- KENNEDY, J. A.; FERRIER, J.; HARBERTSON, J. F.; PEYROT DES GACHONS, C.; 2006: Analysis of tannins in red wine using multiple methods: correlation with perceived astringency. *Am. J. Enol. Vitic.* **57**, 481-485.
- KOUNDOURAS, S.; KANAKIS, I.; DROSSOU, E.; KALLITHRAKA, S.; KOTSERIDIS, Y.; 2013: Effects of postveraison water regime on the phenolic composition of grapes and wines of cv. Agiorgitiko (*Vitis vinifera* L.). *J. Int. Sci. Vigne Vin* **47**, 115-128.
- KOUNDOURAS, S.; MARINOS, V.; GKOLIOTI, A.; KOTSERIDIS, Y.; 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.
- KOUSSISSI, E.; PATERSON, A.; PIGGOTT, J. R.; 2003: Sensory flavour discrimination of Greek dry red wine. *J. Sci. Food Agric.* **83**, 797-808.
- KYRALEOU, M.; KALLITHRAKA, S.; KOUNDOURAS, S.; CHIRA, K.; HAROUTOUNIAN, S.; SPINTHIPOPOULOU, H.; KOTSERIDIS, Y.; 2015: Effect of vine training system on the phenolic composition of red grapes (*Vitis vinifera* L. cv. 'Xinomavro'). *J. Int. Sci. Vigne Vin* **49**, 71-84.
- KYRALEOU, M.; KALLITHRAKA, S.; THEODOROU, N.; TEISSEDE, P.; KOTSERIDIS, Y.; KOUNDOURAS, S.; 2017: Changes in tannin composition of Syrah grape skins and seeds during fruit ripening under contrasting water conditions. *Molecules* **22**, 1453.
- KYRALEOU, M.; KOUNDOURAS, S.; KALLITHRAKA, S.; THEODOROU, N.; PROXENIA, N.; KOTSERIDIS, Y.; 2016a: Effect of irrigation regime on anthocyanin content and antioxidant activity of *Vitis vinifera* L. cv. Syrah grapes under semiarid conditions. *J. Sci. Food Agric.* **96**, 988-996.
- KYRALEOU, M.; KOTSERIDIS, Y.; KOUNDOURAS, S.; CHIRA, K.; TEISSEDE, P.; KALLITHRAKA, S.; 2016b: Effect of irrigation regime on perceived astringency and proanthocyanidin composition of skins and seeds of *Vitis vinifera* L. cv. Syrah grapes under semiarid conditions. *Food Chem.* **203**, 293-300.
- LINGUA, M. S.; FABANI, M. P.; WUNDERLIN, D. A.; BARONI, M. V.; 2016: From grape to wine: Changes in phenolic composition and its influence on antioxidant activity. *Food Chem.* **208**, 228-238.
- MONAGAS, M.; GOMEZ-CORDOVES, C.; BARTOLOME, B.; LAUREANO, O.; RICARDO DA SILVA, J.; 2003: Monomeric, Oligomeric, and Polymeric Flavan-3-ol Composition of Wines and Grapes from *Vitis vinifera* L. cv. Graciano, Tempranillo, and Cabernet Sauvignon. *J. Agric. Food Chem.* **51**, 6475-6481.
- NICULCEA, M.; MARTINEZ-LAPUENTE, L.; GUADALUPE, Z.; SÁNCHEZ-DÍAZ, M.; AYESTARÁN, B.; ANTOLÍN, M.; 2015: Characterization of phenolic composition of *Vitis vinifera* L. 'Tempranillo' and 'Graciano' subjected to deficit irrigation during berry development. *Vitis* **54**, 9-16.
- PAJOVIĆ-ŠČEPANOVIĆ, R.; WENDELIN, S.; FORNECK, A.; EDER, R.; 2016: Varietal differentiation of grapes cv. 'Vranac', 'Kratosija' and 'Cabernet Sauvignon' from Montenegro according to their polyphenolic composition. *Agric. Forest.* **62**, 223-244.
- PALLIOTTI, A.; GATTI, M.; PONI, S.; 2011: Early leaf removal to improve vineyard efficiency: Gas exchange, source-to-sink balance, and reserve storage responses. *Am. J. Enol. Vitic.* **62**, 219-228.
- PELEG, H.; GACON, K.; SCHLICH, P.; NOBLE, A.; 1999: Bitterness and astringency of flavan-3-ol monomers, dimers and trimers. *J. Sci. Food Agric.* **79**, 1123-1128.
- PETROPOULOS, S.; KANELLOPOULOU, A.; PARASKEVOPOULOS, I.; KOTSERIDIS, Y.; KALLITHRAKA, S.; 2017: Characterization of grape and wine proanthocyanidins of Agiorgitiko (*Vitis vinifera* L. cv.) cultivar grown in different regions of Nemea. *J. Food Comp. Anal.* **63**, 98-110.
- PLUMB, G. W.; DE PASCUAL-TERESA, S.; SANTOS-BUELGA, C.; CHEYNIER, V.; WILLIAMSON, G.; 1998: Antioxidant properties of catechins and proanthocyanidins: effect of polymerisation, galloylation and glycosylation. *Free Rad. Res.* **29**, 351-358.

- RINALDI, A.; JOURDES, M.; TEISSEDE, P.; MOIO, L.; 2014: Preliminary characterization of Aglianico (*Vitis vinifera* L. cv.) grape proanthocyanidins and evaluation of their reactivity towards salivary proteins. *Food Sci. Technol.* **164**, 142-149.
- ROCKENBACH, I.; GONZAGA, L.; RIZELIO, V.; GONÇALVES, A. E. S. S.; GENOVESE, M.; FETT, R.; 2011: Phenolic compounds and antioxidant activity of seed and skin extracts of red grape (*Vitis vinifera* and *Vitis labrusca*) pomace from Brazilian winemaking. *Food Res. Int.* **44**, 897-901.
- SHELLIE, K.; 2011: Interactive effects of deficit irrigation and berry exposure aspect on Merlot and Cabernet Sauvignon in an arid climate. *Am. J. Enol. Vitic.* **62**, 462-470.
- WEIDNER, S.; POWALKA, A.; KARAMAĆ, M.; AMAROWICZ, R.; 2012: Extracts of phenolic compounds from seeds of three wild grapevines-comparison of their antioxidant activities and the content of phenolic compounds. *Int. J. Mol. Sci.* **13**, 3444-3457.
- YILMAZ, Y.; TOLEDO, R.; 2004: Major flavonoids in grape seeds and skins: Antioxidant capacity of catechin, epicatechin, and gallic acid. *J. Agric. Food Chem.* **52**, 255-260.
- YILMAZ, Y.; GÖKSEL, Z.; ERDOĞAN, S. S.; ÖZTÜRK, A.; ATAK, A.; ÖZER, C.; 2015: Antioxidant activity and phenolic content of seed, skin and pulp parts of 22 Grape (*Vitis vinifera* L.) cultivars (4 common and 18 registered or candidate for registration). *J. Food Process. Pres.* **39**, 1682-1691.

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