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, Kouississa 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', 'Babice' from Croatia (Čurko et al. 2014), 'Nebbiolo', 'Nasמטה', 'Aglianico' etc. from Italy (Bordiga et al. 2011, Rinaldi et al. 2014), 'Vranac', 'Kratošija' from Montenegro (Pajović-Sćepanović 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 (Čurko 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 contacted in five Greek red wine grape varieties Vitis vinifera L. 'Mavrotragano' (IVC 40210), 'Mandilaria' (IVC 7300), 'Kotsifali' (IVC 6446), 'Agiorgitiko' (IVC 102) and 'Xinomavro' (IVC 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 Creta, 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⁻¹) and then monomeric anthocyanins were determined by high-performance liquid chromatography according to previous method (Kallitharakia 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⁻¹ 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⁻¹ f.w. of seeds or mmol Trolox berry⁻¹. 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>
<thead>
<tr>
<th>Variety</th>
<th>°Brix</th>
<th>Total acidity</th>
<th>pH</th>
<th>Berry weigh</th>
<th>% seeds/berry</th>
<th>% skins/berry</th>
<th>% flesh/berry</th>
<th>skin/flesh</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mavrotragano</td>
<td>24.3 ± 0.2 a*</td>
<td>3.8 ± 0.2 bc</td>
<td>3.7 ± 0.1 a</td>
<td>1.6 ± 0.1 c</td>
<td>6.4 ± 0.5 a</td>
<td>5.4 ± 0.4 d</td>
<td>88.2 ± 1.0 b</td>
<td>0.062 ± 0.01 c</td>
</tr>
<tr>
<td>Mandilaria</td>
<td>20.8 ± 0.3 c</td>
<td>4.0 ± 0.3 bc</td>
<td>3.6 ± 0.1 a</td>
<td>2.3 ± 0.2 ab</td>
<td>4.9 ± 0.5 b</td>
<td>6.6 ± 0.5 c</td>
<td>88.5 ± 1.0 b</td>
<td>0.074 ± 0.01 c</td>
</tr>
<tr>
<td>Kotsifali</td>
<td>22.1 ± 0.9ab</td>
<td>4.6 ± 0.5 b</td>
<td>3.6 ± 0.1 a</td>
<td>2.4 ± 0.1 a</td>
<td>2.7 ± 0.1 d</td>
<td>6.3 ± 0.3 c</td>
<td>90.9 ± 0.3 a</td>
<td>0.070 ± 0.01 c</td>
</tr>
<tr>
<td>Agiorgitiko</td>
<td>22.1 ± 0.2 b</td>
<td>3.5 ± 0.2 c</td>
<td>3.8 ± 0.1 a</td>
<td>2.0 ± 0.1 b</td>
<td>3.8 ± 0.2 c</td>
<td>10.6 ± 0.9 a</td>
<td>85.6 ± 1.1 c</td>
<td>0.124 ± 0.01 a</td>
</tr>
<tr>
<td>Xinomavro</td>
<td>22.2 ± 0.3 b</td>
<td>6.1 ± 0.2 a</td>
<td>3.3 ± 0.0 b</td>
<td>1.9 ± 0.1 b</td>
<td>2.7 ± 0.1 d</td>
<td>8.7 ± 0.5 b</td>
<td>88.7 ± 0.6 b</td>
<td>0.098 ± 0.01 b</td>
</tr>
</tbody>
</table>

* 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⁻¹. 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⁻¹) 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⁻¹), followed by 'Mavrotragano' (3.8 g tartaric acid L⁻¹) and 'Mandilaria' (4 g tartaric acid L⁻¹). Although 'Kotsifali' had the second highest value of TA (4.6 g tartaric acid L⁻¹), 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 and skin flesh⁻¹, 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⁻¹ and % flesh/berry⁻¹. In previous studies, smaller berry size results to higher relative contribution of seeds and skins 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⁻¹ f.w.) as a concentration of fresh weight of seeds or skins and the total values in berries PPTb (mg catechin berry⁻¹) as content per berry and PPTbg (mg catechin g berry⁻¹) as concentration per g berry⁻¹ are present in Fig. 1. Although in the four of the five varieties the contribution of seeds in berry mass was 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⁻¹ 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⁻¹ f.w.) and also
the second highest concentration of seed tannins (19.8 mg·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 depended 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, 'Agioritikiko' showed the lower concentrations of tannins in seeds (14.4 mg·g⁻¹ f.w.) and skins (2.2 mg·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 (Nicolcea 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 mg catechin·berry⁻¹ and a level higher with 41.6 %, 63.2 %, 62.1 % and 64.5 % of 'Mavrotragano', 'Kotsifali', 'Agioritikiko' and 'Xinomavro', respectively (Fig. 1). A lower content of PPTb was observed in 'Xinomavro' berries with 1.4 mg catechin·berry⁻¹. When tannins were expressed as concentration of mg catechin per g berry the PPTbg of 'Mandilaria' was estimated at 1.7 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', 'Agioritikiko' and 'Xinomavro', respectively. No significant differences in PPTb and PPTgb were observed among 'Kotsifali', 'Agioritikiko' and 'Xinomavro'. These results are in agreement to previous findings that wines from 'Mandilaria' appeared to be more astringent compared to those produced from 'Agioritikiko' 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 Mlv·g⁻¹ fresh weight (f.w.) of skins, mg Mlv per berry, the content per berry and mg Mlv per g berry, the concentration per g berry. TAnth in skins fresh weight ranged from 3.7-12.6 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 Mlv values, 1.6 mg·berry⁻¹, 35 % higher than 'Mavrotragano', which was as a consequence of the small berry size of this variety (Table). TAnth from 'Agioritikiko' and 'Kotsifali' grapes did not differ significantly when expressed in fresh weight of skins, but 'Kotsifali' had lower content in berries than 'Agioritikiko'. It was observed that concentrations in g·berry⁻¹ in 'Mavrotragano', 'Mandilaria' and 'Agioritikiko' 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 'Agioritikiko' grapes (Kallithraka et al. 2009).

It has been stated that wines produced from 'Agioritikiko' grapes had a smooth mouth-feel and lower astrignency, whereas wines from 'Xinomavro' grapes were rated more astringent (Kallithraka et al. 2011, Koussissi et al. 2003). In the present study 'Agioritikiko' 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 astrignent 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 mmol Trolox·g⁻¹ f.w. and 0.04 to 0.08 mmol Trolox·g⁻¹ f.w., respectively (Fig. 3).
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⁻¹ f.w.) and in 'Mavrotragano' for the skin tannin extracts (0.08 mmol Trolox·g⁻¹ 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 'Agioritiko' 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 berry size. 'Xinomavro' was the variety with the lowest anthocyanin content expressed either as mg·fresh weight⁻¹ or mg·berry⁻¹. 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⁻¹ and % flesh·berry⁻¹ 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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