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Comparative study on the polyphenols content and antioxidant activity of local wine grape varieties from the island of Paros, Greece

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Abstract

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Six local wine grape varieties grown in island of Paros (Greece) have been studied for evaluating and comparing the polyphenols content and their antioxidant properties. Total phenols content ranges from 768 to 1903 mg (GAE)/kg fresh weight, NFP content ranges from 120.2 to 685.1 mg (GAE)/kg, FP content ranges from 551.4 to 1364.2 mg (GAE)/kg, and F-3-ols content ranges from 131.6 to 363.1 mg (CE)/kg. The white grape variety Maloukato is characterized from the higher total phenols content (1903 mg GAE/kg), while the red grape variety Mandilaria is characterized from the lowest (768 mg GAE/kg). The antiradical activity DPPH ranges from 3.14 to 5.59 μmol Trolox/g fresh weight, while the antioxidant activity FRAP ranges from 1.74 to 3.77 μmol AEE/g. The white grape variety Maloukato is characterized from the higher antiradical activity DPPH, while the red grape variety Vapsa is characterized from the higher antioxidant activity FRAP. High correlation coefficients between he estimated antioxidant activity and the total polyphenols content was found. The grapes cultivation with high polyphenols content may be regarded as principal criterion for producing wines enriched in polyphenols.

Keywords: DPPH activity; FRAP assay; grapes; phenolic fractions; polyphenols

Introduction

The free radicals are strong oxidisers which damage the basic biological molecules (proteins, lipids), destroy the proceeding of the normal biochemical process in the human cells and induce the occurrence of many diseases (Halliwell & Gutteridge, 2015; Al-Waili et al., 2017).

Epidemiological and laboratory investigations demonstrate that the oxidative destruction of biomolecules can be protected by using endogenousand exogenous antioxidants (Hudson, 2012; Mancuso et al., 2012; Rom & Aviram, 2016). Natural polyphenols are components of many fruits and vegetables, affecting the color and their taste, while exercising potent antioxidant and antimicrobial properties (Alesiani et al., 2010).

Grape and its products are rich source of phenolic antioxidants, and are intensively studied as foods or as dietary polyphenols in health promotion and the prevention of diseases (Zhu et al., 2015; Singh et al., 2016; Walery, 2017).

Greece is a country with rich traditions in grape and winemaking whose quality is highly appreciated on the local and export market. The polyphenols contained in them are characterized by high antioxidant and health effect (Makris et al., 2003; Gougoulias, 2009; Gougoulias et al., 2015).

Although there are several studies on the polyphenols content and antioxidant activity of Greek grapes, however yet there are limited reports for the local grape varieties of the island Paros.

The objective of the present study is to investigate and compare the content of polyphenols and their antioxidant action, six local grape varieties which are cultivated on the islands of the Aegean, under the environmental conditions of the island Paros, with purpose to advising winemaking, and to promoting the varietal character of the respective local wines.

Materials and Methods

Experiment

The experiment taken place in four non-irrigated vineyards in the island of Paros in 2016. The vineyards A; B; C and D, located at latitude 37°08′86″ N and longitude 25°24′53″E; 37°11′73″ N and 25°24′56″E; 37°10′58″N and 25°24′56″E, 37°08′53″N and 25°24′45″E, respectively. Each vineyard includes an area about 0.2 ha. The region is characterized by a temperate mediterranean-type climate with mild winters and dry summers. Average winter temperature 13.5°C, average summer temperature 24.5°C and average annual precipitation 480 mm. Three white local grape varieties (Monemvasia, Maloukato and Aidani) and three red local grape varieties (Mandilaria, Aidani and Vapsa) grown (Table 2). The planting of the grape variety maloukato took place the 1915, the distance between adjacent plants in the rows was two meters and between rows also was two meters. For the other grape varieties the planting took place the 1975, the distance between adjacent plants in the rows was 1.7 m, while between rows was 2.2 m. In the vineyard (A) the white variety Monemvasia and red variety Mandilariais grown,during the growing season 30 kg fungicide (brimstone), 1500 ml glyphosate 36% and 105 kg nitrogen per hectare, were added. In the vineyard (B) the white variety Maloukato and the red variety Aidaniis grown,during the growing season 30 kg fungicide (brimstone) per hectare,were added. In the vineyard (C) the white variety Aidani is grown, during the growing season 30 kg fungicide (brimstone), 1500 ml glyphosate 36% and 105 kg nitrogen per hectare,were added. In the vineyard (D) the red variety Vapsa is grown,during the growing season 30 kg fungicide (brimstone) per hectare, were added.

Preparation of methanol extracts

The grapes were collected in the phase of technological maturity from which 100 healthy berries from each grape variety were randomly selected for chemical analysis. The grape extracts were obtained after twofold treatment of 20 g sample with 20 ml 60% methanol solution after one hour storage at dark and room temperature. The collected extracts after centrifugation (filtration) were brought to 50 ml with aqueous methanol and used for chemical analysis (Meyer et al., 1997; Yi et al., 1997).

Methods of Analyses

Grape analysis

Total phenols (TP) contents were determined with the Folin-Ciocalteu reagent according to the method by (Singleton and Rossi, 1965) and were expressed as gallic acid equivalent (GAE)/kg fresh weight. The non-flavonoid phenols (NFP) were determined with the Folin-Ciocalteu reagent after the removal of flavonoid phenols (FP) with formaldehyde according to the method of (Kramling & Singleton, 1969). FP content was determined as a difference between the content of TP and NFP.

The total flavanols (F-3-ols, catechins and procyanidins) were assayed using *p*-DMACA (p-dimethylaminocinnamaldehyde) reagent according to the method of (Li et al., 1996) and were presented as catechin equivalent (CE)/kg fresh weight.

The anthocyanins (AC) were determined according to the method of (Ribereau-Gayon and Stonestreet, 1965), slightly modified by (Burns et al., 2000) by following the change in colour at different pH. They were presented as mg malvidin-3-glicoside/kg fresh weight.

The antiradical activity of the methanol extracts was determined according to the method of (Brand-Williams et al., 1995) applying the stable radical 2,2′-diphenyl-1-picrylhydrazyl (DPPH). The activity was expressed in μmol Trolox (synthetic vitamin E)/g fresh weight.

The ferric reducing antioxidant power (FRAP) was evaluated according to the method of (Benzie and Strain, 1999) and was expressed as ascorbic acid equivalent (AAE) in μmol/g fresh weight.

Soil analysis

Soil was analyzed using the following methods which are referred by Page (1982).

Organic matter was analyzed by chemical oxidation with 1 mol L^{-1} $K_2Cr_2O_7$ and titration of the remaining reagent with 0.5 mol L^{-1} FeSO4. Soil pH and (EC), Electrical conductivity measured in the extract (1 part soil: 5 parts H2O).

Inorganic nitrogen was extracted with 0.5 mol L^{-1} $CaCl_2$ and estimated by distillation in the presence of MgO and Devarda's alloy, respectively. Available P forms (Olsen P) was extracted with 0.5 mol L^{-1} $NaHCO_3$ and measured by spectroscopy. Exchangeable form of potassium was extracted with 1 mol L^{-1} CH_3COONH_4 and measured by flame Photometer (Essex, UK).

Available forms of Mn, Zn, and Cu were extracted with DTPA (diethylene triaminepentaacetic acid 0.005 mol L^{-1} + $CaCl_2$ 0.01 mol L^{-1} + triethanolamine 0.1 mol L^{-1}) and measured by atomic absorption. The samples were analyzed by Atomic Absorption (Spectroscopy Varian Spectra AA 10 plus, Victoria, Australia), with the use of flame and air-acetylene mixture (Varian, 1989).

Statistical analysis

The experiment was as a completely randomized design with four replications. Data analysis was made using the MINITAB (Ryan et al., 2005) statistical package. Analysis of variance was used to assess treatments effect. Mean separation was made using Tukey's test when significant differences (P = 0.05) between treatments were found.

Results and Discussions

The soil of vineyards at depth 0-30 cm is characterized as Sandy Loam (SL). Table 1 shows the chemical properties of the soil of vineyards before from the beginning of crops at depth (0-30) cm. While, Table 2 shows chemical characteristics of berry juice of the cultivars examined, on the stage of technical maturity.

Total phenols content in the white grape varieties which were studied, ranges from 904 to 1903 mg GAE/kg fresh weight (Table 3), while in the red grape varieties ranges from 768 to 1624 mg GAE/kg fresh weight. The white grape variety Maloukato is characterized from the higher total phenols content, equal with 1903 mgGAE/kg fresh weight, while the red grape variety Mandilaria is characterized from the lowest total phenols content, equal with 768 mg GAE/kg fresh weight.

Table 2. Chemical characteristics of berry juices of the cultivars examined

Grape varieties	pH	Brix degrees	Total acidity (g tartaric acid /L)
White			
Monemvasia	4.53 ± 0.22	19.3 ±0.64	2.85 ± 0.05
Maloukato	4.36 ±0.22	21.8 ± 0.94	2.70 ±0.03
Aidani	4.20 ±0.21	18.2 ±0.66	2.63 ± 0.05
Red			
Mandilaria	4.18 ±0.21	15.4 ±0.45	2.25 ± 0.03
Aidani	4.32 ± 0.22	20.6 ±0.71	3.38 ±0.07
Vapsa	4.25 ±0.21	18.5 ±0.58	2.70 ±0.05

Data represent average means and SD, (n) = 4

The white grape variety Aidani, is grown on the island of Santorini, showed lower total phenols content (500.1 mg GAE/kg) compared to our results, while the red grape variety Mandilaria, is grown also on the island of Santorini, showed higher total phenols content (1000.5 mg GAE/kg) compared to our results (Anastasiadi et al., 2010).These results, confirm the variation in the total phenols content in different areas, even for the same grape varieties.

Similar results to ours, on the total phenols content, were also observed for some white and red grape varieties grown in Hebei Province, China and in region Davis, California of the United States (Du et al., 2012; Liang et al., 2014). Studies other authors they showed, that red grape varieties compared to white varieties are characterized by higher total phenols content, but this depends from the cultivar and from the agronomic variables (Kanneretal., 1994; Yi et al., 1997; Marinova et al., 2005; Martínez-Gil et al., 2017).

Table 1. Soil chemical properties at the beginning of crops

Properties	Soil			
	Vineyards (A)	Vineyards (B)	Vineyards (C)	Vineyards (D)
Texture	Sandy loam	Sandy loam	Sandy loam	Sandy loam
pH	8.44 ± 0.22	8.37 ±0.20	7.61 ±0.20	8.01 ±0.20
EC, (dS/m)	0.20 ±0.01	0.19 ±0.01	0.20 ±0.01	0.30 ±0.02
$CaCO_3$ (%)	3.0 ±0.16	21.9 ±0.98	0.56 ±0.02	1.11 ±0.04
Organic matter (%)	1.37 ±0.06	1.88 ±0.07	1.37 ±0.05	0.91 ±0.04
N-inorganic (mg/kg)	35.0 ±4.75	49.0 ±5.21	35.0 ±3.90	49.0 ±5.74
K-exchangeable (mg/kg)	177.8 ±8.82	217.3 ±9.76	167.9 ±5.40	128.4 ±6.40
Na-exchangeable (mg/kg)	71.3 ±3.50	89.7 ±4.30	161.0 ±8.10	96.6 ±4.24
CEC (cmol/kg)	14.0 ± 1.20	15.91 ±1.32	10.1 ±0.98	12.1 ±1.10
P-Olsen (mg/kg)	56.6 ±4.80	60.1 ±5.10	10.5 ±1.12	7.6 ±0.98
Cu-DTPA (mg/kg)	4.1 ±0.10	5.7 ± 0.17	18.2 ±0.90	7.1 ±0.45
Zn-DTPA (mg/kg)	17.4 ±0.82	36.1 ±2.10	22.3 ±1.08	17.7 ±0.76
Mn-DTPA(mg/kg)	1.6 ± 0.10	3.2 ±0.92	8.5 ± 1.06	3.6 ±0.08

Data represent average means and SD, (n) = 4

Table 3. Content of Total phenols (TP), Non-flavonoid (NFP), Flavonoid (FP) phenols, Total flavanols (TF) and Anthocyanins (AC) in grapes of the cultivars examined

Grape varieties	TP	NFP	FP	TF	AC
	mg(GAE)/kg			mg (CE)/kg	mg(m.3.gl.)/kg
White					
Monemvasia	924±36.9d	323.4±14.7b	600.6±28.6cd	164.9±6.8c	–
Maloukato	1903±76.1a	685.1±23.6a	1217.9±48.7b	363. l±15.1a	–
Aidani	904±39.3d	352.6±19.6b	551.4±26.3d	172.5±7.5c	–
Red					
Mandilaria	768±28.4e	120.2±6.7d	647.8±29.4c	131.6±5.1d	477.8±22.3c
Aidani	141 l±52.5c	240.0±10.4c	1171.0±48.8b	216.0±9.0b	717.4±29.9b
Vapsa	1624±50.7b	259.9±11.3c	1364.2±47.1a	225.0±10.7b	896.0±37.3a

In each column different letters indicate significant differences according to the Tukey's test ($P = 0.05$), $n = 4$.

Non-flavonoid phenols content (as equivalent to gallic acid) in the white grape varieties ranges from 323.4 to 685.1 mg GAE/kg fresh weight, while in the red grape varieties ranges from 120.2 to 259.9 mg GAE/kg fresh weight. The white grape varieties which were studied are characterized by higher non-flavonoid phenols content in comparison with the red grape varieties (Table 3). The comparison of the content of NFP in respect to the total phenols content in the white grape varieties shows that they represent from 34 to 39% of TP, while in the red grape varieties represent from 16 to 18% of TP. The NFP fraction consists mainly of gallic, hydroxybenzoic, coumaric, caffeic, ferulic, cinnamic and other acids, and their derivatives (Downey et al., 2006; Lorrain et al., 2011). These compounds affect the taste in grapes and the antioxidant properties.

Flavonoid phenols content (equivalent to gallic acid) in the white grape varieties ranges from 551.4 to 1217.9 mg GAE/kg fresh weight, while in the red grape varieties ranges from 647.8 to 1364.2 mg GAE/kg fresh weight. The comparison of the content of FP in respect to the total phenols content in the white grape varieties shows that they represent from 60 to 65% of TP, while in the red grape varieties represent from 82 to 85% of TP. From the six grape varieties which were studied, the red grape variety Vapsa characterized by higher flavonoid phenols content, equal with 1364.2 mg GAE/kg fresh weight. The established trend in the variation of the fractional composition of phenols under the action of climatic factors is observed also by other authors, even in the same variety (Martínez-Gil et al., 2017).

Although flavanols are within the flavonoid composition, we studied individually. Total flavanols content (as equivalent to catechin) in the white grape varieties ranges from 164.9 to 363.1 mg(CE)/kg fresh weight, while in the red grape varieties ranges from 131.6 to 225.0 mg/kg fresh weight. The white grape variety Maloukato characterized by the higher total flavanols content, equal with 363.1 mg (CE)/kg fresh weight (Table 3).

Similar results to ours, for the red grape varieties, on the total flavanols content has been reported in the red grape variety Carignan grown in different areas of the Maule Valley, Chile (Gutiérrez-Gamboa et al., 2018). Furthermore, these results showed lower values compared with some white and blue grape varieties grown to South Moravia, Czech Republic (Balik et al., 2008).Some authors reported that flavanols synthesis in grapes is favored by the cooler temperatures of the warmest month (Martínez-Gil et al. 2017).

Anthocyanins content (equivalent to malvidin-3-glicoside) in the red grape varieties which were studied ranges from 477.8 to 896.0 mg (malv.-3-gl.)/kg fresh weight. The grape variety Vapsa characterized by the higher anthocyanins content (896 mg malv.-3-gl./kg fresh weight, while the grape variety Mandilaria by the lowest (Table 3).

Our results, compared to those reported by other authors for the same grape varieties grown in other regions of Greece, showed higher total anthocyanins content for grape variety Aidani and lower total anthocyanins content for the grape varieties Mandilaria and Vapsa (Kallithraka et al., 2005). But, these results are consistent with previous studies, which showed grape varieties Aidani and Vapsa to be richer in total anthocyanins, compared with other Hellenic local varieties (Xinomavro, Mavrokountoura, Pardala, Papadiko, etc.) reporting values of 545, 394, 563, and 479 mg/kg of total anthocyanins, respectively (Kallithraka et al., 2005; Mylona et al., 2013).

Similar results on the total anthocyanins content has been reported for some grape varieties grown to southern Serbia (Mitić et al., 2012), but our results showed higher values compared with some grape varieties are grown to Tekirdağ, Turkey (Orak, 2007), and lower values compared with some autochthonous varieties are grown to Andalusia, Spain (Guerrero et al., 2009).

The antiradical activity DPPH• in the grape varieties which were studied ranges from 3.14 to 5.59 μmol Trolox/g fresh weight (Table 4). The white grape variety Maloukato-show higher antiradical activity compared to the red grape varieties studied. The order of antiradical activity DPPH• of the varieties studied are the following: white grape variety Maloukato > red variety Vapsa, red variety Aidani > red variety Mandilaria, white variety Monemvasia, and white variety Aidani (Table 4). The antioxidant activity in the grape varieties which were assayed by the FRAP reagent depends also from the grape variety and ranges from 1.74 to 3.77 μmol AEE/g fresh weight (Table 4). From the grape varieties which were studied, the red grape variety Vapsa show higher antioxidant activity FRAP, while the white grape variety Aidani show lower the antioxidant activity FRAP (Table 4).

Table 4. Antiradical activity and ferric reducing antioxidant power (FRAP) in grapes of the cultivars examined

Grape varieties	Antiradical activity DPPH	Antioxidant activity FRAP
	μmol Trolox/g F.W.	μmol AAE/g F.W.
White		
Monemvasia	3.57±0.18c	1.89±0.09cd
Maloukato	5.59±0.26a	3.27±0.13b
Aidani	3.14±0.15d	1.74±0.07b
Red		
Mandilaria	3.40±0.18cd	2.07±0.08c
Aidani	4.05±0.18b	3.28±0.15b
Vapsa	4.17±0.17b	3.77±0.15a

In each column different letters indicate significant differences according to the Tukey's test (P = 0.05), n = 4.

These results showed higher values of antiradical and antioxidant activity, compared with other native Hellenic and international varieties that can be encountered in the Hellenic vineyard, including Xinomavro, Agiorgitiko, Merlot, Cabernet Sauvignon and Syrah reporting values of 1.45, 1.75, 1.64, 2.14 and 2.08 μmol Trolox/g respectively, and 0.69, 0.88, 0.80, 1.15 and 1.13 μmol AAE/g, respectively (Kallithraka et al., 2009). In addition, for red grape variety Mandilaria, our results showed similar values to those reported by other authors for the same variety grown in the island Crete (Kallithraka et al., 2009).

Also, our results was in agreement with other scientific data, which they report a good antioxidant activity to the grapes (Balik et al., 2008; Gismondi et al., 2017). The antioxidant activity of the grapes depends from the specific influence exerted by each one of their single components of the phenolic compounds and the synergy between of the individual compounds. In addition, our results confirm the hypothesis that the polyphenols in the grape from the island of Paros, due to the antiradical and antioxidant properties are the reason for reducing the risk from many human diseases.

A linear regression analysis was performed to determine the correlation between the total phenols content and the corresponding antioxidant activities in the various grape varieties. High correlation coefficients were found between antiradical activity DPPH and the total phenols content ($R^2 = 0.8363$, $y = 0.0017x + 1.7902$), as well as between antioxidant activity FRAP and total phenols content ($R^2 = 0.7871$, $y = 0.0017x + 0.5579$), n = 24, ($p < 0.05$). High correlation coefficients between he estimated antioxidant activity and the total phenols content in the various grape varieties was also presented and by other authors (Balik et al., 2008).

Conclusions

The study presented herein provided valuable data with regard the influence of grape variety on the total phenols content and phenolic fractions, in six important local wine grape varieties which are cultivated on the islands of the Aegean, Greece.

The total phenols content is closely related with the antioxidant activity determined, of the local grape varieties which were studied. The variability found on the polyphenols content between of the grape varieties confirms that biosynthesis of polyphenols depends from genetic factors and the climatic conditions of region.

The high total phenols content and consequently the powerful antioxidant activity of the grapes studied, they urge the use of these varieties, due to the established biological properties of polyphenols, which are important essential for protection of human health. Therefore, the grapes cultivation with high total phenols content may be regarded as principal criterion for producing wines enriched in polyphenols.

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