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QUALITY CHARACTERISTICS, POLYPHENOL PROFILE AND ANTIOXIDANT CAPACITY IN RED, ROSÉ AND WHITE MONOVARIETAL WINES FROM IONIAN ISLANDS OF GREECE

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ABSTRACT

Background. Wine is one of the oldest traditional alcoholic beverages consumed worldwide. In addition, red and white wines are an excellent source of antioxidant compounds. Numerous studies have focused on the analysis of phenolic acids and flavonoids of several wines originating from organic and conventional cultivars growing grapes. However, to the authors best knowledge, there are no research papers dealing with the phenolic content of wines made on the Ionian Islands so far.
Materials and methods. The amount of total phenolics, total flavonoids, total anthocyanins, total tannins, the concentrations of individual phenolic compounds, as well as the antioxidant capacity, were used synergistically to assess the compositional profile of eleven monovarietal wines.
Results. As expected, red wines exhibited the strongest antioxidant profile. Vertzami was the richest variety in total phenols and antioxidant capacity, followed by the Avgoustiatis variety and Mavrodaphne variety. Regarding white wines, Thiako white had the highest total phenolic values, while Robola rosé contained a similar total phenolic content as the white varieties. A highly positive correlation between the antioxidant capacity and polyphenol composition expressed as total phenolic, total flavonoid, total anthocyanin and total tannin content, was observed. Last, gallic acid and tyrosol were two of the major phenolic compounds detected in all tested wines.
Conclusion. Our findings revealed that the amounts of phenolic contents and antioxidant activity vary significantly in different types of wines. The observed differences could be related to a range of variables such as geographical origin, aging, climate and the vinification techniques.

Keywords: wine, polyphenols, antioxidant activity, color, quality control

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INTRODUCTION

During the last decades, scientific interest has focused on natural antioxidant compounds which are found in food, fruits, vegetables and wine. Several epidemiological studies have demonstrated that the high level of these natural compounds can reduce the risk of chronic diseases such as cardiovascular diseases, cataract, diabetes and neurodegenerative illnesses, as well as cancer (Arvaniti et al., 2019a; Fragopoulou et al., 2020; Gharras, 2009; Nemzer et al., 2021; Shahidi and Ambigaipalan, 2015).

These compounds can inhibit free radical formation by reducing or donating hydrogen to other compounds. Among them, phenolic compounds are the most popular due to their widely known antioxidant capacities, while there are also major components of color, flavor and aroma (Liang et al., 2021). Two major categories of phenolic compounds are phenolic acids and flavonoids. Regarding flavonoids, the most widely studied representative classes are flavanols, flavonols, flavones, and anthocyanidins (Arvaniti et al., 2019a; Gharras, 2009; Shahidi and Ambigaipalan, 2015).

Grapes and wines are an excellent source of phenolic compounds as has been reported by many researchers (Chen et al., 2022; Gouvinhas et al., 2018; Han et al., 2017; Hosu et al., 2014; Mota et al., 2018). This fact is a possible explanation of the *French paradox*, which refers to the fact that France is a country with low incidence of coronary heart disease and mortality, despite French people having a diet high in fat and being heavy smokers (Renaud, 1992). Italy, France and Spain are the main three wine producing countries in Europe, whereas Greece is one of the top twenty wine producing countries.

In the literature, numerous studies have focused on the qualitative and quantitative analyses of phenolic acids and flavonoids in different varieties of red and white wines originating from organic and conventional cultivars growing grapes, while fewer data are available for rosé wine varieties (Feliciano et al., 2009; Li et al., 2009; Lucena et al., 2010; Minussi et al., 2003; Ozkan et al., 2006; Paixao et al., 2007). The most often applied methods for the determination of phenolics and flavonoid compounds in wines are via colorimetric assays (Feliciano et al., 2009; Giacosa et al., 2021; Han et al., 2017; Hosu et al., 2014; Li et al., 2009; Minussi et al., 2003; Mitrevska et al., 2020; Paixao et al., 2007). However, several studies have used chromatographic analytical methods to identify high molecular mass polyphenolic compounds (Chen et al., 2022; Feliciano et al., 2009; Lorrain et al., 2013; Radovanovic et al., 2012; Souza et al., 2018; Stój et al., 2022; Tzanova et al., 2020).

To the best of our knowledge, the content of phenolic compounds has ranged from a few thousands mg gallic acid equivalents (GAE) L^{-1} for red and white wines to several GAE L^{-1} (Baiano et al., 2014; Cimino at al., 2007) for rosé wine (Minussi et al., 2003). The predominant phenolic compounds that have been identified are resveratrol, quercetin, rutin and catechin (Chen et al., 2022; Lorrain et al., 2013; Souza et al., 2018; Tzanova et al., 2020). The color, grape cultivar, bottling time, geographic origin, cultivation practices and harvest season seem to affect the concentrations of phenolic compounds (Benucci, 2020; Carneiro et al., 2020; Gabriele et al., 2018; Ranaweera et al., 2021; Recamales et al., 2006; Setford et al., 2017; Silva Padilha et al., 2018; Souza et al., 2018).

Furthermore, the antioxidant capacity of wines has been widely estimated using different protocols. 1,1-diphenyl-2-picrylhydrazyl radical assay (DPPH), 2,2-azino-di-(3-ethylbenzothialozine-sulphonic acid) assay (ABTS), ferric ion reducing antioxidant power assay (FRAP), cupric ion reducing capability assay (CUPRAC) and oxygen radical absorbance capacity assay (ORAC) are some of the assays that have been widely applied in selected wine varieties (Buyuktuncel at al., 2014; Li et al., 2009; Mitrevska et al., 2020; Romanet et al., 2021; Silva Padilha et al., 2018; Stasko et al., 2008; Stratil et al., 2008). Due to the different experimental conditions used in these assays, no single test can fully characterize the profile of each sample. As a result, their combination is recommended to obtain a sufficient picture of the antioxidant capacity of wines (Arvaniti et al., 2019a).

So far, most of the published data have focused on grape varieties cultivated in South and West European countries such as Italy, Spain and Portugal (Cimino et al., 2007; Feliciano et al., 2009; Giacosa et al., 2021; Loizzo et al., 2013; Paixao et al., 2007). The polyphenolic composition and antioxidant capacity of Greek wines have been examined by few previous studies (Arnous et al., 2001; Kallithraka et al., 2001; 2006;

Roussis et al., 2008; Sakkiadi et al., 2001). Most of them have focused on specific Greek grape varieties such as Agiorgitiko, Mandilaria, Liatiko, Asirtiko and Xynomauro that are cultivated on the mainland. It should be mentioned that the phenolic content of common local wines made from the Ionian Islands has never been analyzed in the past.

Thus, the main objectives of this study were to investigate the total polyphenols content, the concentrations of individual phenolic compounds, as well as the antioxidant capacity of eleven (red, rosé and white) monovarietal wines produced on the Ionian Islands. Physical and chemical parameters (pH, concentration of ethanol, content of total and volatile acidity in g L^{-1}, and density in g L^{-1}) and the content of total and free sulphur dioxide were determined in all examined samples. Color characteristics of selected red, rosé and white varieties were measured. The amount of total phenolics, total flavonoids, total anthocyanins and total tannins in whole wines were determined by spectrophotometric methods. The qualitative and quantitative analyses of selected phenolic compounds were determined using a reversed phase high pressure liquid chromatography (HPLC) method. Lastly, the antioxidant capacity was estimated in the examined wine samples using two different methods (ABTS and DPPH assay). To the best of our knowledge, with the exception of Robola white variety and Vertzami red variety, it is the first time that wines grown on the Ionian Islands have been characterized.

MATERIALS AND METHODS

Chemicals and reagents

Gallic acid was supplied by Merck (Darmstadt, Germany), quercetin was obtained from Alfa Aesar (Karlsruhe, Germany), whereas ascorbic acid and trolox (6-hydroxy-2,5,7,8-tetramethylchroman--2-carboxylic acid) were purchased from Sigma-Aldrich (St. Louis, USA). Folin-Ciocalteu reagent was purchased from Merck (Darmstadt, Germany). 2,2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Sigma-Aldrich (St. Louis, USA). The ABTS reagent [2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid ammonium salt)] was supplied by TCI (Gurgaon, India). Sodium nitrite and aluminium chloride were obtained from Merck (Darmstadt, Germany); sodium hydroxide, sodium bisulfite and potassium persulfate were supplied by Sigma-Aldrich (St. Louis, USA); and sodium carbonate was purchased from Riedel-de Haën (Seelze, Germany). Methanol, ethanol, acetic acid and hydrochloric acid were obtained from Merck (Darmstadt, Germany). Sulfuric acid solution, iodine solution and thiosulfate solution were purchased from Technologia Difusion Iberia (Barcelona, Spain). Standards chemicals such as gallic acid, chlorogenic acid, catechin, tyrosol, quercetin and resveratrol were purchased from Extrasynthese (Genay, France). Stock solutions were used to prepare regularly working standard solutions for calibration curves. Milli-Q grade water was purified by an ultrapure water system (Millipore Direct-Q--UV, Bedford, MA, USA), whereas distilled water was provided by a CFL Water Distillation Units (Dublin, Ireland). All chemicals and solvents were analytical grade (>99%).

Wine samples

Eleven commercial dry wines (4 red, 1 rosé and 6 white) originating from conventional cultivars of growing grapes were collected in the present study. Wine samples had been produced from 2015 to 2017 in different regions of the Ionian Islands. Detailed information about the geographical origin, grape variety and vintage of all examined wines is listed in Table 1. All samples were stored at 4°C in the dark and analyzed immediately after opening. An aliquot of examined wine samples was filtered through pharmaceutical cotton prior their analysis.

Determination of quality characteristics

pH, alcohol, density, total and volatile acidity of eleven tested wine samples were determined using a Fourier--transform infrared spectroscopy (FTIR) wine analyzer (Thermo Scientific Nicolet iS5). The free and total sulphur oxide contents were determined in the whole wine using a wine analyzer TDI ENO 20. Specifically, for free sulphur oxide assessment, a 20 mL aliquot of wine sample was transferred to a 50 mL beaker and mixed with 2 mL H_2SO_4 25% (v/v). The total amount of free sulphur oxide was automatically and rapidly titrated against iodine solution 0.02 N. Regarding the total sulphur oxide measurement, 20 mL of wine sample and 2 mL NaOH 5 N were added into a beaker

Table 1. Origin, grape variety, color and vintage of all the Ionian Island wines examined

Wine samples	Location	Cultivar	Color	Vintage
Kakotrigis	Corfu	Cacotrigi	white	2017
Goustolidi	Kefalonia	Goustolidi	white	2017
Muscat	Kefalonia	Moscato	white	2016
Robola White	Kefalonia	Robola	white	2016
Thiako White	Ithaca	Thiako White	white	2017
Tsaousi	Kefalonia	Tsaousi	white	2017
Robola Rosé	Zante	Robola	rosé	2016
Thiako Red	Ithaca	Thiako Red	red	2017
Mavrodaphne	Kefalonia	Mavrodafni	red	2015
Vertzami	Lefkada	Vertzami	red	2016
Avgoustiatis	Zante	Augustiatis	red	2017

of 50 mL. The reaction solution was incubated for 10 min at room temperature and then 4 mL H_2SO_4 was added. The total amount of sulphur oxide was directly quantified on titration analyzer equipment. Every five samples, a calibration solution was measured.

Wine color analysis

The CIE *L*a*b** color space was obtained using a colorimeter Lovibond PFXi-195 Series (Tintometer Ltd., UK). The following color coordinate: lightness (*L**), red-green (*a**) and yellow-blue (*b**) were obtained. *L** parameter ranges from black = 0 to white = 100. A negative value of *a** refers to a green color, while a positive one represents the red-purple color. Positive and negative values of *b** parameter indicate yellow and blue color, respectively. The chroma (*C*) and hue angle (*H*°) were calculated according to Rolle and Guidoni (2007).

Determination of polyphenol profile

Total phenolic content. The amount of total phenolics in wine samples was measured based on our previous published Folin-Ciocalteu assay (Arvaniti et al., 2019b). Absorption was measured at 750 nm using a Unicam Helios UV-Vis spectrophotometer and the results were expressed as mg GAE L^{-1}. Standard solutions of gallic acid were prepared at five concertation levels ranging from 50 to 1000 mg L^{-1}. A control sample was prepared by replacing the wine sample with an aqueous ethanol solution 12% v/v.

Total flavonoid content. The amount of total flavonoids in tested wines was measured according to published work by Arvaniti et al. (2019b). Absorption was read at 510 nm using a Unicam Helios UV-Vis spectrophotometer and the results were expressed as mg quercetin equivalents (QE) per L. Standard solutions of quercetin were prepared at five concentration levels ranging from 50 to 500 mg L^{-1}. A control sample was prepared by replacing the wine sample with an aqueous ethanol solution 12% v/v.

Total anthocyanins content. The amount of total anthocyanins in studied wines was measured according to the described method by Ribereau-Gayon and Stonestreet (1965). Absorption was read at 520 nm using a Unicam Helios UV-Vis spectrophotometer. To calculate the anthocyanins' content as mg L^{-1}, the difference of absorbance between a sample prepared with distilled water and a sample prepared with sodium bisulfite was calculated and the obtained value was multiplied by a factor equal to 875. Distilled water was used for the spectrophotometer's zeroing, while no standard was used in this method.

Total tannins content. The amount of total tannins in the wine samples was determined according to Ribereau-Gayon and Stonestreet (1966). Absorption was measured at 550 nm using a Unicam Helios UV--Vis spectrophotometer. The difference of absorbance between a sample heated and a sample not heated was calculated. The obtained value was multiplied by a factor equal to 19.35 and the results were expressed as g L^{-1}. Distilled water was used for the spectrophotometer's zeroing, while no standard was used in this method.

Determination of individual phenolic compounds

Millex®-SV syringeless PVDF filter membranes (5.0 µm; Millipore, Bedford, USA) were used for the filtration of wine samples prior to instrumental analysis. The qualitative and quantitative analyses for the determination of individual phenolic compounds were carried out using an Agilent 1260 HPLC system interfaced with an Agilent 1260 diode array detector (DAD). Chromatographic separation was performed by Zorbax Eclipse Plus C18 (4.6 × 250 mm, 5 µm) column from Agilent. Data were acquired with the OpenLAB CDS Chemstation software package (Agilent Technologies). A previously chromatographic method was applied with some modifications (Malovana et al., 2001). More specifically, a gradient elution program with water:acetic acid:methanol (88:2:10, v/v/v; solvent A) and water:acetic acid:methanol (8:2:90, v/v/v; solvent B) as binary mobile phase mixture at a flow rate of 1 mL/min was used. The gradient elution started with 0% (v/v) solvent B and increased linearly to 15% in 15.0 min, then to 50% in 10.0 min and to 70% in 10.0 min, held at 70% for 10.0 min, reverted to 100% in 10 min and re-equilibrated for 10.0 min (from 55.0 to 65.0 min) at 0% solvent B for a total run time of 65.0 min. Sample and standards were injected into the column with full-loop injection (20 µL) and the column temperature was set at 30°C. The detection wavelength was 280 nm for gallic acid, catechin and tyrosol, while for resveratrol and chlorogenic acid was 320 nm and 365 nm for quercetin. A mixture of target analytes standard solution was prepared in MeOH. Figure 1 shows a chromatogram

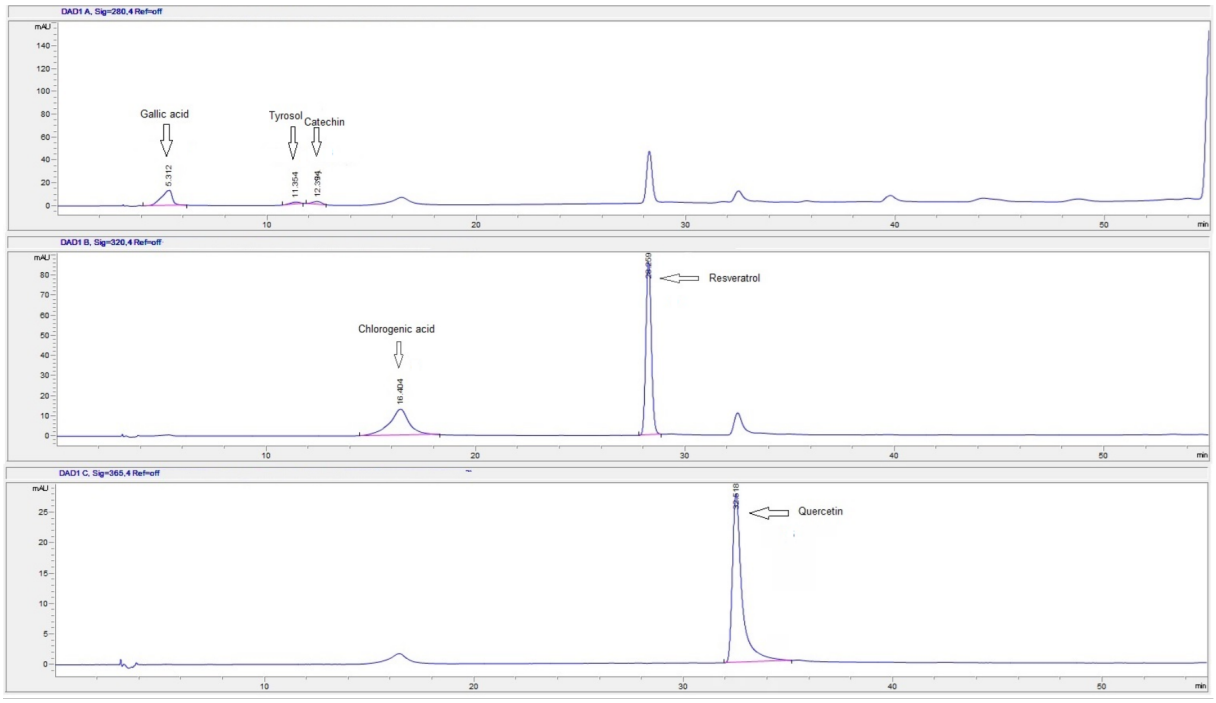

Fig. 1. An HPLC characteristic chromatography of a mixture standard solution

Table 2. Analytical parameters of the applied analytical method

Target compounds	Instrumental linear range μM	Calibration curve	Instrumental correlation coefficient (r^2)
Gallic acid	5−300	$y = 10.73x - 42.94$	0.9975
Tyrosol	25−150	$y = 1.001x + 13.87$	0.9994
Catechin	25−150	$y = 1.445x - 4.901$	0.9958
Chlorogenic acid	5−150	$y = 21.69x - 115.4$	0.9722
Resveratrol	5−100	$y = 31.08x - 17.93$	0.9992
Quercetin	1−100	$y = 17.76x - 6.384$	0.9987

of a mixture standard solution at a final concentration level of 50 μM for each target compound. A calibration curve was constructed for each analyte and all standards gave linear calibration curves. Each wine sample was analyzed and injected into HPLC-DAD in triplicate and the average concentrations of these three measurements are reported below. Detailed information about the analytical methods is presented in Table 2.

Determination of antioxidant capacity

DPPH assay. The free radical scavenging activity of wines was determined according to the published method of Brand-Williams et al. (1995) with some modifications. The absorbance was measured at 492 nm using an ELISA plate reader (TECAN Sunrise) and the absorbance of remaining DPPH was recorded. The percentage inhibition of the DPPH radical scavenging activity for each sample was determined by making use of the following formula:

$$I, \% = \frac{\mathrm{Abs}_c - \mathrm{Abs}_s}{\mathrm{Abs}_s}$$

where:

I – the DPPH inhibition, %,

Abs_c – the absorbance of control sample,

Abs_s – the absorbance of the examined wine sample at the end of the reaction at t = 30 min in each case.

In addition, standard solutions of ascorbic acid (AsA) were prepared at eight different concentration levels ranging from 0.1 to 1.2 mM and a calibration curve based on the % DPPH radical scavenging activity of known concentrations of AsA was constructed. The samples were qualificated using the linear regression analysis and the results were expressed as mM AsA. The control sample was prepared replacing wine sample by an aqueous ethanol solution 12% v/v.

ABTS assay. ABTS assay was determined based on a previously published method by Re et al. (1999) with some slight modifications. ABTS radical cation ($ABTS^{\bullet+}$) was produced by mixing 7 mM of ABTS ammonium salt solution and 2.45 mM of potassium persulfate aqueous solution. The solution was kept in the dark, at room temperature for 12–16 h before use. The $ABTS^{\bullet+}$ solution was diluted with ethanol to obtain an absorbance of 0.70 ±0.02 at 734 nm. Results were expressed as mM trolox equivalent (TE). Standard solutions were prepared at concentrations ranging from 0.05 to 0.60 mM. The control sample was prepared by replacing the wine sample with an aqueous ethanol solution 12% v/v.

Statistical analysis

The Kolmogorov-Smirnov test was used to assess normal distribution. Normally distributed variables were expressed as mean ±standard deviation. Variables with normal distribution at baseline were analyzed with the one-way analysis of variance (ANOVA) test. Multiple pairwise comparisons were performed using the Bonferroni correction. Pearson correlation coefficients were calculated between normally distributed variables. The SPSS statistical software package (version 18) was used for all the statistical calculations. All the reported p-values were compared with a significance level of 5%.

RESULTS AND DISCUSSION

Physicochemical characteristics

The average values of the physical and chemical parameters (pH, concentration of ethanol, content of total and volatile acidity in g L^{-1}, and density in g L^{-1}) of eleven different wine varieties are presented in Table 3, as well as their content of free and total sulphur dioxide. As can be observed, the determined percentage of ethanol in white wines ranged between 11.55% (Robola white variety) and 13.53% (Thiako white variety), 11.40% for Robola rosé variety, and up to 13.64% for Vertzami red variety. The mean value of ethanol percentage of white wines was lower than that of red wines (12.34 ±0.75 versus 13.28 ±0.25, $p < 0.000$). The total and volatile acidity values of different wines were ranged in acceptable quality limits. The mean value of total acidity was significantly different between white and red wines (5.08 ±0.42 versus 5.86 ±0.84, $p < 0.000$), while the mean value of volatile acidity was not different between white and red wines (0.50 ±0.53 versus 0.69 ±0.27, $p = 0.157$). The Thiako variety, both red and white, showed higher volatile acidity values. The mean pH value of white wines was significantly lower than that of red wines (3.38 ±0.09 versus 3.47 ±0.14, $p = 0.006$), and the pH measurements ranged from 3.24 (Robola variety; white wine) to 3.68 (Mavrodaphne variety; red wine). The total SO_2 amount ranged between 13 and 116 mg SO_2 L^{-1} in all examined samples (Table 3). In the rosé variety, the total SO_2 was found up to 23 mg L^{-1}, while the mean value of total SO_2 of four red wines was 55.25 ±31.88 and for 6 white wines, it was 71.0 ±37.8. No significant difference was achieved ($p = 0.46$) between red and white wines, although in most cases, the content of total SO_2 was found to be higher in white wine varieties

Table 3. Physicochemical characteristics of eleven wine samples examined

Wine samples	Ethanol* %	pH*	Density* g L^{-1}	Total acidity* g L^{-1}	Volatile acidity* g L^{-1}	Free SO_2** mg L^{-1}	Total SO_2** mg L^{-1}
White wines							
Kakotrigis	12.24 ±0.010	3.51 ±0.010	0.9910 ±0.0001	**4.6 ±0.040**[a]	0.44 ±0.010	12	49
Goustolidi	12.14 ±0.032	3.35 ±0.010	0.9929 ±0.0001	**4.7 ±0.010**[a]	**0.26 ±0.00**[a]	13	64
Muscat	13.07 ±0.041	3.37 ±0.010	**0.9916 ±0.0001**[a]	5.3 ±0.060	**0.25 ±0.010**[a]	10	79
Robola White	**11.55 ±0.018**[a]	3.24 ±0.0040	0.9927 ±0.0001	5.2 ±0.10	**0.24 ±0.010**[a]	36	105
Thiako White	13.53 ±0.016	3.45 ±0.010	0.9913 ±0.0001	5.8 ±0.1	1.65 ±0.011	3	13
Tsaousi	**11.6 ±0.01**[a]	3.39 ±0.01	**0.9915 ±0.0001**[a]	4.9 ±0.02	0.17 ±0.00	15	116
Rosé wine							
Robola Rosé	11.40 ±0.02	3.27 ±0.01	0.9898 ±0.0001	5.0 ±0.03	0.31 ±0.01	n.d.	23
Red wines							
Thiako Red	13.17 ±0.010	3.32 ±0.010	0.9925 ±0.0001	6.9 ±0.040	1.10 ±0.012	8	23
Mavrodaphne	12.97 ±0.018	3.68 ±0.010	0.9947 ±0.0001	**4.6 ±0.040**[a]	0.70 ±0.010	6	71
Vertzami	13.64 ±0.050	3.43 ±0.010	**0.9916 ±0.0001**[a]	5.9 ±0.050	0.53 ±0.01	4	35
Avgoustiatis	13.33 ±0.017	3.47 ±0.0040	0.9940 ±0.0001	6.0 ±0.04	0.40 ±0.011	34	92

*Values are reported as means ±standard deviation (SD) of six replicates carried out in each variety.
**Values are the average of two measurements.
n.d. – not detected.
For all variables with the same letter, the difference between the means is not statistically significant.

than red wines. This observation occurs as red wines do not need further addition of sulphur dioxide because of their natural content of antioxidants from their skins and stems during fermentation (Coetzee et al., 2013).

Color evaluation

One of the most important sensory attributes of wines is the color. It should be mentioned that customers have a preference for wines with a nice color. For this reason, it is crucial to produce wines with an attractive color and high nutritional value as well. In the present study, the wine color features *L**, *a**, *b**, Chroma and Hue angle of eleven wine varieties were determined by colorimetric experiments, and the results are given in Table 4. The results showed that all white wine varieties (except for Thiako white variety) exhibited negative *a** values indicating less red color and more green color; and high *b** values which mean a strong yellow tone. Moreover, their *L** values were higher than 80.89 (Thiako white variety), demonstrating high lightness of white wines. Calculated low *C* values indicated weak chroma for almost all white wine, with exception of Thiako white variety which showed *C* values of 27.81. Regarding red wines, a dark color was observed due to their low lightness (*L**) values and their high values of *a** and *b** parameters. *C* values were calculated in the range from 47.18 to 63.21 for Avgoustiatis variety and Thiako red variety, respectively. According to the results, *L** values were found in the range from 9.95 (Avgoustiatis variety) to 34.98 (Thiako red variety); whereas the high *a** values were determined from 43.99 to 49.61, and between 17.04 and 39.17 for *b** values (Table 4). Rosé variety showed *L**, *a** and *b** values equal to 80.00, 9.75 and 23.24, respectively. In addition, hue angles (H°) estimated for red wines ranged from 21.17° (Avgoustiatis variety) to 38.29° (Thiako red variety), 67.24° for rosé variety, and between 86.21° (Thiako white variety) and 106.69° (Tsaousi variety) for white wine variety. These values classified the type of color for each analyzed wine sample. More specifically, 0° is responsible for red-purple, 90° for yellow, 180° for bluish-green and 270° for blue chroma.

Table 4. Color characteristics of white, rosé and red wines from Ionian Islands

Wine samples	*L**	*a**	*b**	*C*	*H*°
White wines					
Kakotrigis	92.84	–0.21	9.69	9.69	91.24
Goustolidi	93.17	–2.14	10.95	11.16	101.06
Muscat	96.08	–2.09	7.21	7.51	106.17
Robola White	96.22	–1.48	5.08	5.29	106.24
Thiako White	80.89	1.84	27.75	27.81	86.21
Tsaousi	95.92	–1.46	4.87	5.08	106.69
Rosé wine					
Robola Rosé	80.00	9.75	23.24	25.20	67.24
Red wines					
Thiako Red	34.98	49.61	39.17	63.21	38.29
Mavrodaphne	14.71	48.52	25.36	54.75	27.59
Vertzami	10.74	45.20	18.51	48.84	22.27
Avgoustiatis	9.95	43.99	17.04	47.18	21.17

*L**, *a**, *b**, *C*, and *H*°, are color parameters: lightness/darkness, red/green, blue/yellow, chroma and hue angle, respectively.

Levels of polyphenols content

The total phenolic (TP), total flavonoid (TF), total anthocyanin (TA) and total tannin (TT) content of studied wines varieties were determined by spectrophotometric methods (Table 5). Among the examined wine varieties, Vertzami and Thiako white variety exhibited the highest polyphenol content for red and white wine varieties, respectively. As expected, TP content found to be higher in red wines than white varieties (2002 ±693 versus 496 ±105 mg GAE L^{-1}, $p < 0.000$), while rosé variety contained a similar TP content to the white varieties (539 ±62 mg GAE L^{-1}, $p = 1.0$). It is well-known that the red wines are rich in TP. The presence of high TP content in red varieties could be explained by the greater grape skin and seed contact time and due to the applied temperature for their fermentation process (Giacosa et al., 2021; Han et al., 2017; Li et al., 2009; Mitic et al., 2010; Nemzer et al., 2021; Paixao et al., 2007; Stój et al., 2020). Also, our results fell within the range reported for other countries. In addition, it is interesting to note that similar levels of TP have been reported in the literature for Robola variety (Kallithraka et

Table 5. Total phenolics, flavonoids, anthocyanins and tannins content; and DPPH and ABTS assay results of the wine samples examined

Wine samples	Total phenolics mg GAE L^{-1}	Total flavonoids mg QE L^{-1}	Total anthocyanins mg L^{-1}	Total tannins g L^{-1}	DPPH	ABTS
					mM AsA	mM TE
White wines						
Kakotrigis	527 ±16.8[a]	638 ±14[a]	n.d.	16.4 ±0.45[a]	4.26 ±0.16	1.62 ±0.08
Goustolidi	563 ±19.7[a]	738 ±39[a]	n.d.	28.3 ±2.64[a,b]	4.66 ±0.01	1.85 ±0.06
Muscat	320 ±3.7	245 ±8	n.d.	8.28 ±0.45[a]	3.61 ±0.08	1.00 ±0.02
Robola	468 ±13.0[a]	331 ±8[a]	n.d.	6.93 ±0.37[a]	3.80 ±0.14	1.00 ±0.77
Thiako White	643 ±29.5[a]	837 ±34[a,b]	n.d.	26.5 ±1.65[a,b]	5.76 ±0.16	1.92 ±0.01
Tsaousi	456 ±13.1[a]	513 ±15[a]	n.d.	21.8 ±0.55[a,b]	3.25 ±0.18	1.50 ±0.02
Rosé wine						
Robola Rosé	539 ±62.0[a]	630 ±53[a]	4.81 ±0.62[a]	14.6 ±1.91[a]	5.64 ±0.38	1.71 ±0.05
Red wines						
Thiako Red	881 ±18.0	1125 ±106[b]	19.7 ±3.09[a]	48.6 ±5.05[b]	9.25 ±0.29	2.10 ±0.12
Mavrodaphne	2258 ±90.0[b]	3325 ±247	79.6 ±8.66[b]	132.6 ±15.1[c,d]	20.6 ±0.19	4.49 ±0.01
Vertzami	2581 ±121.0	3958 ±88	98.0 ±3.71[b,c]	153.8 ±18.9[c]	20.9 ±0.52	4.56 ±0.03
Avgoustiatis	2291 ±69.0[b]	2983 ±184	130.8 ±16.7[c]	117.4 ±10.1[d]	21.2 ±0.21	4.42 ±0.02

Values are reported as means ±standard deviation (SD) of triplicate determination carried out in the whole wines.
Total phenols expressed as gallic acid equivalents – GAE. Total flavonoids expressed as quercetin equivalents – QET. Total anthocyanins expressed as malvidin-3-glucoside – M3G. Ascorbic acid – AsA. Trolox equivalent – TE.
n.d. – not detected.
For all variables with the same letter, the difference between the means is not statistically significant.

al., 2001; Roussis et al., 2008) and Vertzami variety (Kallithraka et al., 2006) grown in the same geographical parts of Greece (Ionian Islands; Kefalonia and Lefkada).

TF content of four red examined wines was higher than that of white varieties (2977 ±1053 versus 531 ±214 mg QE L^{-1}, $p < 0.000$), while for Robola rosé it was 630 ±53 mg QE L^{-1} and similar to white wines ($p = 1.0$).

Regarding the amount of TA, anthocyanins were not detected in any white varieties; while in red varieties 82.3 ±43.7 mg L^{-1} and 4.81 ±0.62 mg L^{-1} were found. Similar results with the present study were observed in a previous study by Li et al. (2009), who determined the TA content in red and white wines. On the other hand, slightly higher levels of TA have been reported by Kallithraka et al. (2006) for other red wine varieties produced in Greece. In addition, among red wines, Vertzami variety and Mavrodaphne exhibited higher concentration levels of TT, ranging up to 153.8 ±18.9 and 117.4 ±10.1 g L^{-1}, respectively. The TT amount in red wines was higher than in white wines (117.8 ±44.1 versus 18.1 ±8.6 g L^{-1}, $p < 0.000$), and in Robola rosé variety, it was 14.6 ±1.9 g L^{-1}.

Comparing the results of the present study with published data for TP and TF content, a variation among wine samples is observed. These differences could be attributed to several factors such as grape variety, environmental factors in the vineyard, climate, soil type, different harvesting and wine processing methods and aging (Buyuktuncel et al., 2014; Li et al., 2009; Paixao et al., 2007).

Table 6. Levels of specific phenolic compounds in wine samples from Ionian Islands, μM (μmol L^{-1})

Phenolic compound	Gallic acid	Chlorogenic acid	Catechin	Quercetin	Tyrosol	Resveratrol
			White wines			
Kakotrigis	10 ±1.2	36 ±1.9	122 ±14.7	n.d.	793 ±40.8	1 ±0.1
Goustolidi	9 ±1.3	15 ±1.0	166 ±12.9	n.d.	555 ±24.6	2 ±0.2
Muscat	73 ±3.8	97 ±1.2	81 ±3.9	n.d.	177 ±3.4	1 ±0.02
Robola White	20 ±0.9	21 ±1.7	119 ±9.5	n.d.	522 ±2.8	2 ±0.1
Thiako White	107 ±2.0	42 ±2.8	226 ±27.9	n.d.	475 ±8.4	2 ±0.1
Tsaousi	16 ±1.9	11 ±0.5	113 ±3.4	1 ±0.03	678 ±45.5	1 ±0.1
			Rosé wine			
Robola Rosé	40 ±0.2	23 ±1.5	115 ±15.8	n.d.	527 ±66.0	2 ±0.2
			Red wines			
Thiako Red	103 ±0.3	27 ±0.8	324 ±13.3	n.d.	798 ±50.1	2 ±0.005
Mavrodaphne	248 ±4.2	60 ±2.7	188 ±2.8	n.d.	934 ±4.2	2 ±0.1
Vertzami	263 ±4.2	65 ±1.4	29 ±0.7	2 ±0.3	1030 ±133.6	3 ±0.4
Avgoustiatis	220 ±1.6	39 ±0.03	n.d.	n.d.	795 ±39.6	1 ±0.1

Values are reported as means ±standard deviation (SD) of triplicate determination carried out in the whole wines.
n.d. – not detected.

Occurrence of specific phenolic compounds

A total of six phenolic compounds and their concentrations are given in Table 6. These examined compounds include two phenolic acids, two flavonoids, one alcohol and one stilbene. Figure 1 shows a chromatogram of a standard mixture solution in which the separation of six target compounds can be seen; whereas Figure 2 presents a chromatogram for a white, rosé and red wine sample. More specifically, the predominant phenolic antioxidants substance for all examined wine samples was tyrosol, followed by gallic acid. Red wines had higher levels of tyrosol (889.4 ±119.7 versus 533.5 ±200.0, $p = 0.001$) and gallic acid (208.5 ±67.2 versus 66.5 ±38.8, $p < 0.000$) than white wines.

It should also be noted that chlorogenic acid appeared to be at the same levels of concentration in all examined wines ($p = 0.411$) with the concentration range of 36 ±1.9 μM (Kakotrigis) to 97 ±1.2 μM (Muscat). Quercetin and resveratrol were found to be below the LOQ in all Ionian Islands wine samples (Table 4). Regarding white wines, the wine sample "Thiako white" showed the highest levels of phenolic compounds, while the "Vertzami" sample exhibited the highest levels for red wine, respectively.

Antioxidant capacity

In the present study, the total antioxidant capacity of wine samples was quantified by DPPH and ABTS assays. These results are summarized in Table 3. In red wines, the scavenging activity against DPPH, expressed as ascorbic concentration, was 17.7 ±5.5 mM AsA and significantly higher than in white wines (4.2 ±0.9 mM AsA ($p < 0.000$)). In rosé wine (Robola rosé variety) 5.64 ±0.38 mM AsA was found. A similar trend of results was observed by performing ABTS assay. Red wines had higher values compared to white ones (3.88 ±1.13 versus 1.47 ±0.39 mM TE, $p < 0.000$). This potent antioxidant capacity of red wines is in accordance with our previous studies, and it can be related mainly to their increased total phenolic compounds content (Fragopoulou et al., 2020; Xanthopoulou et al., 2010).

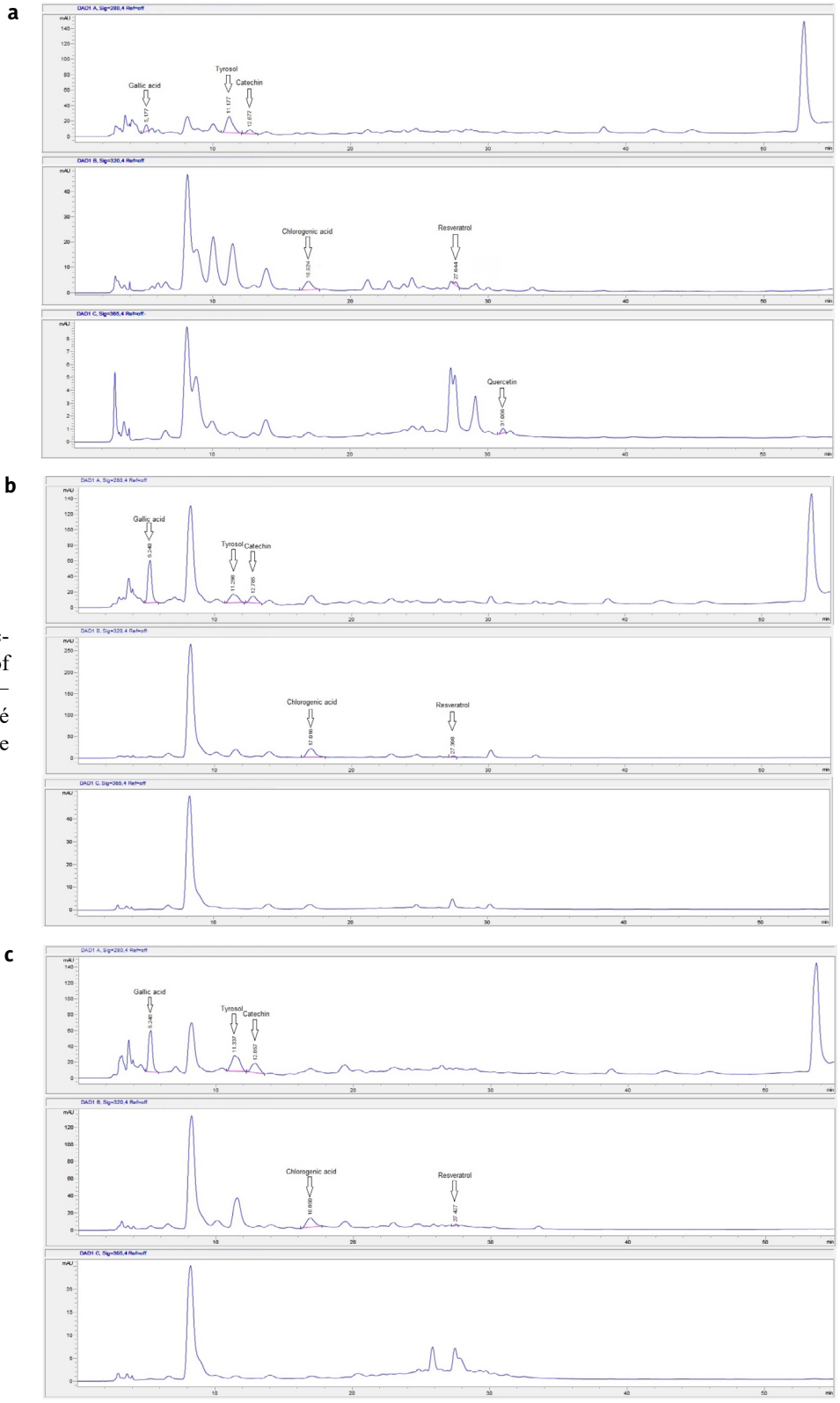

Fig. 2. An HPLC characteristic chromatography of phenolic compounds in: **a** – white wine (variety), **b** – rosé wine (variety), **c** – red wine (variety) sample

Table 7. Correlations of antioxidant activity of wines with polyphenol content and their chemical characteristics

		DPPH	ABTS
Ethanol percent	correlation coefficient	0.678 (0.000)	0.660 (0.002)
pH	correlation coefficient	0.395 (0.042)	0.507 (0.023)
Density	correlation coefficient	0.478 (0.012)	0.373 (0.105)
Total acidity	correlation coefficient	0.407 (0.035)	0.270 (0.250)
Volatile acidity	correlation coefficient	0.618 (0.001)	0.670 (0.001)
Free SO_2	correlation coefficient	–0.345 (0.298)	–0.406 (0.215)
Total SO_2	correlation coefficient	–0.410 (0.210)	–0.389 (0.237)
Total phenolics	correlation coefficient	0.937 (0.000)	0.946 (0.000)
Total flavonoids	correlation coefficient	0.918 (0.000)	0.982 (0.000)
Total anthocyanins	correlation coefficient	0.927 (0.000)	0.673 (0.033)
Total tannins	correlation coefficient	0.784 (0.000)	0.948 (0.000)

Numbers in brackets show the *p* value.

Correlation between antioxidant capacity and polyphenols composition

Correlation analysis of antioxidative parameters of analyzed wine samples with their chemical characteristics and polyphenols content was performed (Table 7). According to the analysis, a significant positive correlation was found between the anti-oxidant capacity (DPPH and ABTS) and the ethanol percentage, pH and volatile acidity, while only DPPH was positively correlated with density and total acidity. In addition, a highly positive correlation was observed between the antioxidant capacity and polyphenol composition expressed as total phenolic (TP), total flavonoid (TF), total anthocyanin (TA) and total tannin (TT) content. These results agree with previous research works which report a high correlation of these methods (Arvaniti et al., 2019a). The aforementioned results also indicated that the antioxidant capacity of examined wines was tightly influenced mainly by their total phenol content, followed by flavonoids and tannins amount, while anthocyanins content played a minor role.

CONCLUSIONS

In the present study, eleven monovarietal dry wines produced on the Ionian Islands were examined. Spectrophotometric, chromatographic and colorimetric methods were synergistically applied to assess their compositional profile. The most remarkable result drawn from this research was that the combination of all applied methods provides critical information on the polyphenol content of wine varieties that have never been analyzed in the past. The strongest antioxidant activity was exhibited by the red wine varieties; a finding that was significantly correlated to the highest level of total phenolic content. HPLC analysis confirmed the occurrence of phenolic compounds such as tyrosol, catechin, gallic acid, and chlorogenic acid. Further investigation is required to determine the effects of geographical origin, environmental factors in the vineyard, climate, soil type, different harvesting and wine processing methods and aging.

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