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Original Article

Assay of Antioxidant Capacity and Phenolic Compounds in some Romanian and Cypriot Wine

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Abstract

Free radicals have an important role in food and in chemical material degradation, contributing to the occurrence of many human health problems, but the antioxidants can considerably delay or prevent the oxidation of easily oxidable substrates. The present research aimed to assess the antioxidant activity, expressed by the presence of polyphenols, flavonols, flavones, anthocyanidins and flavanols, in several Romanian and Cypriot wines. The wine phenolics content was analysed by high-performance liquid chromatograph (HPLC) Shimadzu equipped with two chromatographic columns. Higher concentrations were registered in all red wines. The antioxidant activity quantification was carried out by the DPPH method, a simple and cheap approach based on the absorbance decrease determination of the DPPH radical (2,2-diphenyl- 1- picrylhydrazyl) in the presence of antioxidants. The highest antioxidant activity for white wines was determined at 'Spouriko' for Cypriot wine from 2013 (EC 50 = 1/38) while for Romanian wines, the highest value was found in a 'Tămâioasă românească' (EC50 = 1/58) and for red wines at 'Maratheftiko' wine from 2012 (EC50 = 1/680) and in 'Fetească Neagră' wine from 2014 (EC50 =1/590). This study provides relevant information to consumers and industry alike regarding the beneficial role wine plays for human health. It also can act as a baseline for choosing a certain product, according to its sanogenic potential.

Keywords: antioxidant activity, DPPH method, phenolic compounds, Cypriot wines, Romanian wines

Introduction

There is a worldwide agreement that anthocyanins, flavonols, catechins, and other flavonoids contribute to the wine colour and astringency, while it has also been demonstrated that they scavenge the excess radicals and mitigate oxidative stress. Therefore, they contribute to the anticarcinogenic, antiatherogenic, antiinflammatory, antimicrobial, and antioxidant activities of some fruits (Llaudy *et al*., 2004; Chang *et al*., 2012; Xu *et al*., 2012).

Among natural antioxidants, red wine has attracted particular interest due to a high content of biologically active compounds (Lopez-Velez *et al*., 2003; Tsai *et al*., 2004). The moderate consumption of wine, especially red wine, has also been associated with the reduction in mortality from cardiovascular diseases, an effect known as the "French Paradox" (Renaud and De Lorgeril, 1992). The polyphenolic compounds present in wines, which are known to have a high antioxidant capacity, are involved in several protective activities against some degenerative diseases such as cancers, cardiovascular diseases, chronic – inflammation and thrombosis (Bell *et al*., 2000; Scalbert *et al*., 2005; Majo *et al*., 2008; Sun *et al*., 2009; Xia *et al*., 2010). Hence, the beneficial properties of wines have been mainly interpreted based on the antioxidant properties on the flavonoid fraction, which are related to free radical scavenging (Cao and Prior, 2000). Non-flavonoid compounds are presented mainly in the pulp of the grapes, and the flavonoid compounds are found in the skins, seeds, and stems of grapes (Cotea *et al*., 1985). The phenolic composition of wines is conditioned by the grape variety and by other factors that influence the berry development, such as soil, geographical location, weather conditions (Rotaru *et al*., 2013) or management practices (Bunea *et al*., 2012). Once grapes are crushed, condensation reactions, which involve especially anthocyanins, catechins and procyanidins, take place, resulting in the formation of new

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pigments, which are responsible for wine colour changes. Winemaking techniques also play an important role in the extraction of polyphenols from grapes and in their further stability in wines; the time of maceration and fermentation in contact with the grape skins and seeds, pressing, maturation in oak, fining, and bottle aging influence the phenolic composition of wines (Cotea *et al*., 2010).

The content of phenolic substances and total antioxidant activity of the sets of samples are high correlated as many studies described (Arnous *et al*., 2002; Katalinić *et al*., 2004; Hua *et al*., 2009; Mitić *et al*., 2010). Several in vitro methods have been developed to measure antioxidant capacities of food, beverages and biological samples. The most commonly used antioxidant capacity assays were 1,1-diphenyl-2-picrylhydrazyl radical (DPPH•) assay (Bondet *et al*., 1997); 2,2-azino-di-(3-ethylbenzothialozine-sulphonic acid) (ABTS) assay (Re *et al*., 1999); ferric ion reducing antioxidant power (FRAP) assay (Benzie *et al*., 1996; Pulido *et al*., 2000); cupric ion reducing capability (CUPRAC) assay (Apak *et al*., 2004) and oxygen radical absorbance capacity (ORAC) assay (Cao *et al*., 1996; Naguib, 2000).

The DPPH method is a rapid and simple method for estimating the antiradical activity of foods using the stable free radical 1,1- diphenyl-2-pycrylhydrazyl (DPPH•) by the addition of scavenging compounds. This is one of a few stable and commercially available organic nitrogen radicals and shows a characteristic UV–Vis spectrum with a maximum absorbance close to 515 nm (Saint-Cricq de Gaulejac *et al*., 1999; Da Porto *et al*., 2000; Paixao *et al*., 2007).

All wine samples were analyzed for phenolic compounds content on a HPLC system Shimadzu Prominence 20 series (Castellari *et al*., 2002, Cotea *et al*., 2012).

The aim of this paper was to characterize the free radical scavenging activity using DPPH method (diphenyl-p-picrylhydrazyl radical) and HPLC analysis of phenolic compounds content of some commercial Romanian and Cypriot wines.

Materials and Methods

Samples

The present study has chosen 55 white, rosé and red wine samples (25 Cypriot and 30 Romanian) of different vintages and different areas of Cyprus and Romania. Cypriot wines for 2012 vintage of which 5 white and 10 reds, 15 for 2013 vintage, of which 9 whites and 5 reds. The distribution of analysed Romanian wines is as follows: a white wine vintage 2006, vintage 2011 with 3 whites, one red from 2012, vintage 2013 with 8 whites and 3 reds, vintage 2014 with 10 whites, 1 rosé and 2 reds. All were still wines. All wine samples are presented in Table 1.

The chosen grape varieties are well-known both for the Cypriot and the Romanian red, white and rosé wine-making, there for deemed important for this study.

Reagents

All used reagents have been purchased from Sigma-Aldrich Co (St. Louis, MO, USA). All other chemicals used were of analytical grade.

Determination of antioxidant activity/capacity of wines through diphenyl-p-picrylhydrazyl (DPPH) method

The original procedure (Sánchez-Moreno *et al*., 1995) was modified by using a platform for the antiradical depletion DPPH, made with a Visible or UV-Vis spectrophotometer, multi-plate reader M200 Pro (Tecan Group Ltd., Männedorf, Switzerland) with polymethyl methacrylate well plates. A series of dilution was needed to calculate the result. This dilutions were: Reference solution D0: 9 mL of the DPPH• methanolic solution + 100 µL of MeOH; dilution D1: 1/40 dilution of wine (4 mL of the DPPH• + 100 µL wine); dilution D2: 1/80 dilution of wine (4 mL DPPH• + 50 µL wine); D3: 1/160 dilution of wine (4 mL DPPH• + 25 µL wine); D4: 1/320 dilution of wine (4 mL DPPH• + 12.5 µL wine); D5:1/640 dilution of wine (4 mL DPPH• + 6.25 µL wine). The antioxidant activity was evaluated based on free DPPH• radicals remaining in the medium after the reaction between the methanolic DPPH• solution and the tested samples took place. For each dilution from D0 to D5, the reduction in the absorbance was determined at 515 nm at 0 min. and every 1 min. for 14 min., and every 10 min. until the reaction reaches a plateau in about 1 hour. The antioxidant activity of the wine is thus defined by the dilution of wine required to decrease the initial concentration of DPPH• by 50%: Efficient Concentration = EC50. Under these conditions, the lower EC50 of a tested samples, the higher its antioxidant activity.

HPLC phenolic compounds analysis

For the phenolics content analysis (Castellari *et al*., 2002), the wine samples were processed on a Shimadzu HPLC system consisting of: quaternary pump Shimadzu Prominence LC-20AD with autoinjector SIL-20AC, diode array detector SPD 600 nm, chromatographic system controller CBM connectivity via LAN. The column system was made of a pre Cartridges UHPLC C18 for 4.6 mm ID coupled to columns manufactured by Phenomenex. The elution flow was 0.85 mL min^{-1} and the column compartment was set at 50 °C. The amount of phenolic compounds in the extracts was calculated as mg/L wine using external calibration curves, which were obtained for each phenolic standard.

Statistical analysis

As the data was not normally distributed, Spearman's–Rho (r_s) correlation coefficients were calculated in order to characterize the relationship between antioxidant capacities detected by DPPH assay and phenolics content quantified by HPLC method. Spearman's rank correlation coefficient is a measure of correlation, written in short as the Greek letter *rho(ρ)* or sometimes as r_s. It is a number that shows how closely two sets of data are linked. It only can be used for data that can be put in order, such as highest to lowest. The general formula for r_s is:

$$\rho = 1 - \frac{6\Sigma d^2}{n(n^2 - 1)}$$

where:

d = difference in paired ranks and n = number of cases. The following guide for the absolute value was used: 0.00-0.19 "very weak correlation"; 0.20-0.39 "weak correlation"; 0.40-0.59

"moderate correlation"; 0.60-0.79 "strong correlation"; 0.80-1.0 "very strong correlation", as mentioned in other research studies (Fenercioglu *et al*., 2010; Floegela *et al*., 2011; Harris *et al*., 2011).

All statistics were performed with Microsoft Excel™ 2000. Correlations were established using regression analysis at a 95, 99, and 99.9% significance level. The P-value less than 0.05 were considered statistically significant.

Table 1. The Cypriot and the Romanian analysed wine samples

Sample Code	Colour	Vintage	Grape variety
		Cypriot Wines	
C-12-01	White	2012	'Promara'
C-12-02	White	2012	'Chardonnay'
C-12-03	Red	2012	'Merlot'
C-12-04	White	2012	'Morokanella'
C-12-05	White	2012	'Spouriko'
C-12-06	White	2012	'Xynisteri'
C-12-07	Red	2012	'Maratheftiko'
C-12-08	Red	2012	'Giannoudi'
C-12-09	Red	2012	'Maratheftiko'
C-12-10	Red	2012	'Maratheftiko'
C-13-01	White	2013	'Sauvignon Blanc'
C-13-02	White	2013	'Promara'
C-13-03	White	2013	'Promara'
C-13-04	White	2013	'Morokanella'
C-13-05	White	2013	'Chardonnay'
C-13-06	White	2013	'Spouriko'
C-13-07	White	2013	'Xynisteri'
C-13-08	Red	2013	'Cabernet Franc'
C-13-09	Red	2013	'Morokanella'
C-13-10	Red	2013	'Ntopio Mauro'
C-13-11	Red	2013	'Ofthalmo'
C-13-12	Red	2013	'Maratheftiko'
C-13-13	White	2013	'Xynisteri'
C-13-14	White	2013	'Xynisteri'
C-13-15	Red	2013	'Giannoudi'
		Romanian Wines	
R-07-01	White	2014	'Grasă de Cotnari'
R-07-02	White	2014	'Francușă'
R-07-03	White	2014	'Fetească regală'
R-07-04	White	2014	'Muscat Ottonel'
R-07-05	White	2014	'Tămâioasă românească'
R-07-06	White	2014	'Aligoté'
R-07-07	White	2014	'Sauvignon blanc'
R-07-08	White	2014	'Traminer'
R-07-09	White	2014	'Riesling italian'
R-07-10	Rosé	2014	'Busuioacă de Bohotin'
R-07-11	Red	2014	'Pinot noir'
R-07-12	Red	2014	'Fetească neagră'
R-07-13	White	2014	'Pinot gris'
R-07-14	Red	2014	'Băbească neagră'
R-07-15	White	2013	'Zghihară de Huși'
R-07-16	Red	2013	'Fetească neagră'
R-07-17	White	2011	'Fetească albă'
R-07-18	White	2013	'Fetească albă'
R-02-01	White	2011	'Tămâioasă românească'
R-02-02	White	2011	'Grasă de Cotnari'
R-02-03	White	2013	'Fetească albă'
R-02-04	White	2013	'Francușă'
R-02-05	White	2006	'Tămâioasă românească'
R-02-06	White	2013	'Aligoté'
R-02-07	White	2013	'Sauvignon blanc' Bio
R-02-08	White	2013	'Fetească regală'
R-02-09	White	2013	'Busuioacă de Bohotin'
R-02-10	Red	2013	'Băbească neagră'
R-02-11	Red	2013	'Cabernet sauvignon'
R-02-12	Red	2012	'Cabernet sauvignon'

Results and Discussion

The wines used in this study constituted a quite heterogeneous group, with different grape varieties, with diverse ages and ageing processes, therefore they showed important differences. Antioxidant activity results expressed as EC50 of different types of wines (red wine, white wine and rose wine) determined by the DPPH method are shown in Table 2. The obtained results correlate well with other literature finds: The method applied to samples of red wines shows that the efficient concentration factor EC50 varies approximately from $2.22*10^{-3}$ to $1.66*10^{-3}$. For white wines, the EC50 varies from $1.25*10^{-2}$ to $4*10^{-3}$. (Brand-Williams *et al*., 1995; Saint-Cricq de Gaulejac *et al*., 1999; Da Porto *et al*., 2000).

The obtained EC50 is inversely related to the antioxidant activity of a compound, as it expresses the amount of antioxidant needed to decrease the radical concentration by 50%. The lower EC50, the higher the antioxidant activity of a compound is (Carmona-Jiménez *et al*., 2014). All wines scavenged DPPH• differently. Red wines were more active than whites. This can be attributed to their higher phenolic content.

Among the three wine colour groups, red Cypriot wines showed the highest antioxidant capacity, followed by rosés and whites (Fig. 1). Cypriot wines showed a higher antioxidant capacity than Romanian ones, on average.

Table 2. Effective concentration factor (EC50) for analysed wine samples

Sample Code	Efficient Concentration factor (EC 50)
Cypriot Wines	
C-12-01	3.33E-02
C-12-02	3.13E-02
C-12-03	2.87E-03
C-12-04	4.00E-02
C-12-05	1.89E-02
C-12-06	2.94E-02
C-12-07	1.27E-02
C-12-08	2.99E-03
C-12-09	1.47E-03
C-12-10	2.08E-03
C-13-01	2.44E-02
C-13-02	4.35E-02
C-13-03	4.00E-02
C-13-04	3.57E-02
C-13-05	9.52E-03
C-13-06	2.04E-02
C-13-07	6.25E-02
C-13-08	1.97E-03
C-13-09	2.44E-02
C-13-10	2.63E-02
C-13-11	6.90E-03
C-13-12	2.54E-03
C-13-13	3.13E-02
C-13-14	2.17E-02
C-13-15	2.43E-03
	Continuation (Romanian Wines)→
Romanian Wines	
R-07-01	6.25E-02
R-07-02	6.67E-02
R-07-03	5.88E-02
R-07-04	7.69E-02
R-07-05	3.45E-02
R-07-06	7.69E-02
R-07-07	8.33E-02
R-07-08	2.04E-02
R-07-09	7.69E-02
R-07-10	2.08E-02
R-07-11	1.64E-02
R-07-12	1.81E-03
R-07-13	2.44E-02
R-07-14	4.59E-03
R-07-15	2.86E-02
R-07-16	3.09E-03
R-07-17	5.56E-02
R-07-18	4.35E-02
R-02-01	1.72E-02
R-02-02	2.38E-02
R-02-03	6.25E-02
R-02-04	3.85E-02
R-02-05	2.78E-02
R-02-06	3.23E-02
R-02-07	4.76E-02
R-02-08	5.00E-02
R-02-09	2.04E-02
R-02-10	4.78E-03
R-02-11	2.62E-03
R-02-12	5.24E-03

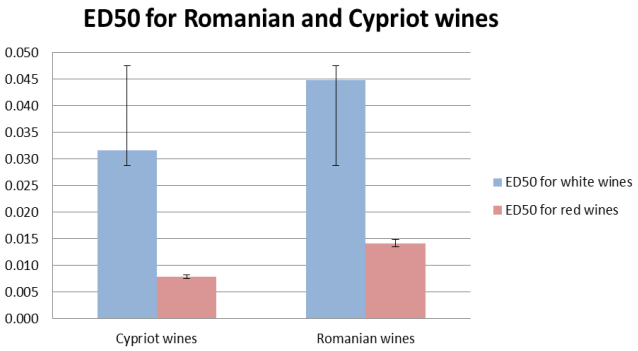

Fig. 1. Comparison of antioxidant capacities measured by DPPH assays, stratified by wine colour and wine nationality (mean and SD)

The HPLC analysis is the method used for the separation and quantification of a large variety of phenolic compounds from wine composition. The HPLC approach achieved 13 components including phenolic acids and flavonoids: gallic acid, protocatechic acid, gentisic acid, vanillic acid, caffeic acid, chlorogenic acid, syringic acid, p-coumaric acid, ferulic acid, salicylic acid, trans-resveratrol, cis-resveratrol, quercitine (Tables 3, 4). The levels of the different compounds found in wine samples are comparable to those reported in literature.

Among Cypriot wines, 'Maratheftiko' has the higher antioxidant activity (Galanakis *et al.*, 2015); our study has reached the comparable result, which confirms that this method can be used in wine analysis. It should be taken into consideration that the wine is "alive", in a continuous transformation, its composition depends first on the terroir, then on the winemaking techniques or on the storage conditions.

In two of the Romanian wines, 'Frâncusă' R-07-02, 'Feteasca regală' R-07-03, but also well-known 'Aligoté' R-07-06, quercitine content was under the detection limit, while other phenolics were well represented. Gallic acid, with anti-fungal and anti-viral properties, has the highest concentration in 'Feteasca neagră' R-07-16, (6315.54 mg L^{-1}) and 'Merlot' C-12-03 (11206.57 mg L^{-1}). Trans-resveratrol has the highest content in 'Merlot' C-12-03 (1593.06 mg L^{-1}) and 'Pinot noir' R-07-11 (544.84 mg L^{-1}). Sources of trans-resveratrol in food include the skin of grapes, blueberries, raspberries, mulberries. Trans-resveratrol provides health benefits, ranging from protection against disease to antiaging properties (Fremont, 2000).

The quantification of the phenolic compounds was performed in order to provide a correlation between DPPH findings and the concentration of the wine samples in phenolic compounds (Table 5). Statistical data underlines the fact that antioxidant capacities by DPPH assay have a strong negative correlation with protocatethic acid (r_s = -0.78, n=55, p<0.001), syringic acid (r_s = -0.73, n=55, p<0.001), trans-resveratrol (r_s = -0.70, n=55, p<0.001) and gallic acid (r_s = -0.66, n=55; p<0.001). A moderate negative correlation was observed between the DPPH assay and quercitine (r_s = -0.52, n=55, p<0.01), n being the number of

Table 3. Quantified phenolic compound in Romanian wine sample (mg/L)

Sample	Gallic acid	Protocatechuic acid	Gentisic acid	Vanillic acid	Caffeic acid	Chlorogenic acid	Syringic acid	*p*-coumaric acid	Ferulic acid	Salicylic acid	*Trans*-resveratrol	*Cis*-resveratrol	Quercitine
R-07-01	45.83	10.89	37.12	31.59	2094.35	0.79	3.01	215.96	663.97	320.22	4.4	1.24	10.28
R-07-02	22.49	12.57	4121.34	31.96	15.15	1.39	12.78	2.19	30.95	14.28	62.09	1.16	
R-07-03	36.92	3.72	6492.35	11.75	14.33	2.98	7.66	3.57	96.25	23.41	7.83	1.46	
R-07-04	47.1	4.97	44.62	9.64	613.48	3.06	1.28	107.71	180.58	94.04	4.88	2.06	6.11
R-07-05	21.58	7.47	19849.9	19.68	42.75	0.86	1.73	7.94	118.18	64	8.21	1.19	1.85
R-07-06	14.55	5.54	5390.19	12.27	5.27	43.46	2.35	2.43	31.03	4.88	61.46	1.4	
R-07-07	19.33	5.53	52.98	12.49	466.42	19.46	1.12	54.38	145.64	81.88	4.76	1.24	2.48
R-07-08	44.18	14.27	59.62	22.69	676.75	4.40	4.44	91.64	31.3	161.34	79.68	0.93	5.78
R-07-09	9	5.51	15977.09	15.08	69.03	2.84	2.12	16.68	70.55	13.45	6.79	1.03	1.31
R-07-10	253.04	50.3	152.91	432.21	2977.93	4.29	25.38	418.9	370.43	11.18	129.47	0.53	2.7
R-07-11	1003.59	52.36	7775.16	935.05	1199.48	0.89	122.78	236.82	321.56	111.77	544.84	0.74	6.89
R-07-12	1041.35	30.62	6263.23	340.99	1037.54	3.69	61.97	258.71	162.81	108.86	331.45	3.35	17.4
R-07-13	40.99	9.29	52.15	98.32	852.87	1.61	23.58	108.67	91.34	88.04	8.5	0.58	6.05
R-07-14	913.32	17.63	7148.38	137.39	387.78	4.01	41.81	145.25	152.04	56.85	169.62	1.14	9.95
R-07-15	272.63	33.28	16346.53	36.65	115.87	1.5	1.63	21.71	46.03	9.13	12.45	0.31	1.18
R-07-16	6315.54	79.41	13686.39	594.48	562.87	28.73	211.1	201.71	45.68	60.53	471.71	0.96	3.54
R-07-17	439.74	28.38	5070.44	48.21	491.33	4.83	6.24	100.98	248.94	18.63	10.28	2.89	7.33
R-07-18	173.38	22.62	5740.75	59.12	66.36	2.88	3.93	24.91	249.85	108.05	7.6	0.64	1.62
R-02-01	675.59	59.13	11506.64	40.86	264.33	5.03	5.32	68.04	129.03	4.16	11.73	0.74	9.57
R-02-02	405.5	33	7367.29	42.55	148.32	5.19	1.77	30.28	178.18	65.38	5.1	0.61	1.64
R-02-03	29.34	9.69	7445.12	39.71	53.43	13.59	2.22	13.8	194.94	108.78	14.7	0.69	1.64
R-02-04	235.14	8.78	2513.45	31.46	365.16	1.05	3.51	83.27	150.3	10.18	7.76	0.63	5.71
R-02-05	261.54	93.13	2149.36	66.73	1613.94	22.37	5.56	389.39	171.05	17.29	66.29	1.14	24.57
R-02-06	422.72	19.24	14.07	34.4	1461.89	4.64	5.16	122.69	261.11	147.12	7.64	0.7	5.26
R-02-07	270.7	15.56	154.32	49.57	106.98	5	2.11	39.91	214.24	47.7	6.31	0.51	1.23
R-02-08	313.15	11.87	5047.24	29.41	387	5.73	1.87	63.13	154.72	18.48	11.41	1.16	4.26
R-02-09	44.59	35.04	95.84	177.21	2579.3	11.31	16.86	128.87	12.18	3.87	8.2	0.82	1.79
R-02-10	571.22	46.85	9815.54	116.69	194.97	8.69	29.52	84.54	90.64	21.1	38.91	0.76	6.99
R-02-11	2443.12	110.85	6912.93	587.76	965.67	8.24	123.1	103.59	49.01	18.6	463.94	0.84	5.83
R-02-12	487.33	37.89	177.19	274.28	1093.18	6.07	46.76	225.31	37.92	3.86	47.12	0.38	1.09

Table 4. Quantified phenolic compounds in Cypriot wine sample (mg/L)

Sample	Gallic acid	Protocatechuic acid	Gentisic acid	Vanillic acid	Caffeic acid	Chlorogenic acid	Syringic acid	*P*-coumaric acid	Ferulic acid	Salicylic acid	*Trans*-resveratrol	*Tis*-resveratrol	Quercitine
C-13-01	30.22	18.05	1517.74	95.31	1.97	1.92	3.02	1.59	87.95	57.73	7.50	0.34	0.75
C-13-02	396.27	14.25	7452.68	42.63	38.77	1.24	4.06	8.04	55.73	6.92	5.95	0.25	1.48
C-13-03	151.12	11.94	15009.12	71.26	29.71	1.29	1.92	3.58	42.35	7.63	19.94	0.35	1.48
C-13-04	388.66	12.14	12238.09	19.43	47.63	1.76	5.56	0.48	125.46	4.77	11.00	0.22	1.01
C-13-05	352.03	46.29	7115.57	97.13	7.97	1.17	8.14	0.80	79.68	13.04	5.55	0.27	2.66
C-13-06	652.94	30.78	12264.82	28.57	20.43	0.72	5.71	3.37	48.96	6.10	38.43	0.47	2.66
C-13-07	484.89	11.61	7670.02	105.73	39.12	0.72	5.53	4.04	78.28	3.75	6.92	0.21	2.23
C-13-08	2246.79	90.20	15526.65	548.58	3.00	0.97	50.33	5.21	11.26	304.77	814.31	24.71	320.29
C-13-09	1369.01	45.49	28.42	43.67	22.11	0.89	2.84	13.28	180.63	22.52	114.44	0.78	1.03
C-13-10	179.81	23.98	24354.68	140.23	18.10	3.71	42.12	0.90	10.08	5.30	234.72	0.70	1.11
C-13-11	4805.33	62.27	11896.78	514.50	40.39	5.78	16.75	2.44	16.51	12.46	177.86	5.47	151.59
C-13-12	2720.72	52.68	32042.01	353.38	77.33	87.70	30.23	3.91	9.22	279.94	1162.87	23.90	149.54
C-13-13	602.27	26.65	13049.35	42.40	63.29	1.43	2.47	15.12	8.53	28.73	69.61	0.42	4.36
C-13-14	286.54	12.82	13704.23	7.14	37.13	3.60	2.16	2.00	47.48	5.75	10.43	0.17	4.36
C-13-15	208.79	34.98	17359.95	703.58	4.69	108.83	102.83	1.90	28.61	118.73	541.50	1.59	142.50
C-12-01	4.81	13.72	7627.52	36.69	392.62	5.12	43.60	1.56	11.06	4.30	12	0.31	4.05
C-12-02	960.59	42.83	7436.28	58.17	14.38	2.30	4.09	8.04	22.82	11.05	15.41	0.65	1.78
C-12-03	11206.57	137.28	63.62	1122.88	80.55	48.50	116.38	5.25	27.52	291.49	1593.06	4.73	69.38
C-12-04	1870.63	30.22	16.94	48.74	12.12	0.82	4.60	0.78	124.62	3.53	7.97	0.12	0.75
C-12-05	1493.20	59.18	8685.72	24.30	119.81	1.10	11.88	1.15	4.91	5.07	127.02	0.51	1.38
C-12-06	999.01	33.04	3224.42	185.94	57.41	2.83	6.20	0.82	61.88	6.05	21.22	0.42	4.16
C-12-07	388.66	12.14	12238.09	19.43	47.63	1.76	5.56	0.48	125.46	4.77	11	0.22	1.01
C-12-08	7324.84	94.62	7877.22	988.15	117.93	3.92	94.28	3.50	4.65	116.25	549.16	5.08	150.79
C-12-09	4795.94	92.40	30530.61	785.10	278.56	124.90	54.44	8.71	26.72	20.93	1320.95	7.73	15.11
C-12-10	8886.82	136.28	27743.89	1326.95	245.20	162.12	100.49	8.41	18.80	752.49	900.89	56.44	148.35

Table 5. Spearmans–Rho coefficient of correlation between antioxidant capacities measured by DPPH assay (EC50) and phenolic compounds of the studied wine

Parameter	Spearmans–Rho coefficient	p-value
EC50	1	-
gallic acid	-0.669	<0.001
protocatechic acid	-0.781	<0.001
gentisic acid	-0.349	>0.01
vanillic acid	-0.212	<0.05
caffeic acid	-0.128	>0.5
chlorogenic acid	-0.302	<0.05
syringic acid	-0.731	<0.001
p-coumaric acid	-0.053	<0.05
ferulic acid	0.385	<0.05
salicylic acid	-0.211	<0.05
trans-resveratrol	-0.707	<0.001
cis-resveratrol	-0.235	<0.05
quercitine	-0.529	<0.01

samples. These findings suggested that phenolic acids are the most important contributor to antioxidant capacity in these wines.

The highest antioxidant activity for Cypriot white wines was determined at 'Spouriko' wine from 2013, while for Romanian wines the highest value was found in a 'Tămâioasă românească' and for red wines at 'Maratheftiko' wine from 2012 and in 'Fetească neagră' wine from 2014, which demonstrated that the antioxidant activity varies with vintage, grape variety and region (Tables 1 and 2).

Conclusions

Red wines showed higher antioxidant activity than white or rosé wines. The Spearmans–Rho statistical analysis revealed that the antioxidant capacities determined by DPPH assay have a strong negative correlation with protocatehic acid, syringic acid, trans-resveratrol and gallic acid and the results presented in this paper can be considered recommendations for consumers who are looking for certain benefits in choosing a wine.

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