

**STUDIES OF TOTAL PHENOL CONTENTS, ANTHOCYANS AND
ANTIOXIDANT ACTIVITY OF SOME GREEK RED WINES**

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ABSTRACT

The study involved 16 red-wine brands produced from more than one grape cultivars originated from different regions of Greece. The total phenol contents ranged from 1360 to 3970 ppm gallic acid equivalents, and their anthocyanins varied from 10 to 428 μM malvidin-3-glucoside equivalents. The antiradical activity, assessed through inhibiting the free stable radical DPPH varied from 2.96 to 14.80 $\mu\text{mol DPPH/ml}$ and the antioxidant capacity determined by the FRAP assay ranged from 9.95 to 26.75 $\mu\text{mols FRAP/ml wine}$. The antioxidant and antiradical activity of the wines tested depended strongly on the total phenolic content. This work showed that Greek red wine is important antioxidant source, a valuable tool for local nutraceutical industries which will make them great competitors in the global market.

INTRODUCTION

The favourable climate and soil conditions of Greece, as part of Mediterranean basin, meet the optimum requirements for growth of a wide spectrum of grapes varieties and production of good quality wines. Grape and wines are an important source of natural antioxidants (Kanner et al., 1994). In particular, red wine has a phenolic content considerably higher than white wine due to winemaking procedure (Frankel et al., 1993; Leighton et al., 1998). Phenol rich foods and beverages contribute to the prevention of several diseases associated with oxidative stress (Muselík et al., 2007). Furthermore, grape seed polyphenolic compounds combined with antioxidant vitamins are currently used with success in nutraceutical industry (Nuttall et al., 1999).

Due to industrial importance of wine antioxidant activities, there is a need for more in-depth-analysis of traditional wines around the globe. The aim of this work was to determine the levels of total phenols, anthocyanins, antioxidants in Greek red wines and measure their antiradical attributes.

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MATERIAL AND METHODS

Sixteen Greek red wines produced from one or more grape varieties were used in our study (Table 1). Samples from wines had been produced from 2004 to 2006 in different wine regions of Greece were used. The total phenol content was determined using the Folin-Ciocalteu assay (Singleton and Rossi, 1965; Badenschneider et al., 1999) and the results expressed as gallic acid equivalents (GAE) or catechin.

The anthocyanins were measured using the method of Ribereau-Gayon and Stonestreet (1965) and expressed in malvidin-3-glucoside equivalents (molar extinction coefficient of 28000). The ferric reducing antioxidant activity was assessed *in vitro* by the FRAP (Ferric reducing/antioxidant power assay) method of Benzie and Strain (1999), using Fe^{3+} -tripyrindyl triazine complex (Fe^{3+} -TPTZ). The antioxidant activity was expressed as $\mu\text{mol/ml}$ wine. The antiradical activity was assessed by bleaching the free stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl) using the method of Brand-Williams et al. (1995). The initial DPPH $^{\cdot}$ concentration was calculated by the equation:

$$A_{515\text{nm}} = 12509 C_{\text{DPPH}} - 2.58 \times 10^{-3}$$

The antioxidant activity of the L-ascorbic acid standard was 11304 $\mu\text{mol/g}$ (Simonetti et al., 1997; Fogliano et al., 1999).

Data were analyzed using the Minitab statistical package. Standard deviation was calculated for all measurements. The linear regression between total phenolic content and antioxidant activity as well between total phenolic content and antiradical activity was examined.

RESULTS AND DISCUSSION

The total phenols content varied between wines. 'Ktima averof' red wine had the highest concentration of total phenols (3820 ppm GAE) while 'Daphnes' the lowest (1360 ppm GAE). The mean concentration of total phenols was 2605 ppm GAE (Table 1). Several researchers have also been observed differences in the phenolic content among grape varieties (Frankel et al., 1995; Simonetti et al., 1997; Burns et al., 2000). The difference in the content of total phenols depends on several factors such as variety, climatic and ecological factors, cultural practises, harvesting method and winemaking procedure.

The content of anthocyanins on red wines fluctuated from 10 to 428 μM malvidin-3-glucoside equivalents. Their quantity at red wine depends on the wine production method and aging. Old wines have lower content from new ones (Mazza, 1995). In our study wines produced on 2006 had greater anthocyanins content than wines produced on 2004 with 'Rapsani' wine to have the greatest concentration.

The antioxidant activity (FRAP) differed between 16 red wines brands (table 2). All wines had high antioxidant activity which reduces Fe. The wine 'methistanes' had the greatest FRAP activity (26.75 μmol FRAP/ml wine) and 'Grand Rose' the lowest (9.95 μmol FRAP/ml wine). Antioxidant capacity of wines is in close relation with their concentration in total and individual phenolic acid (Pulido et al., 2000). In our study there was linear correlation between the amount of total phenols and antioxidant capacity of red wines ($r^2=7822$), (Figure 1).

The antiradical activity of wines was ranged from 2.96 to 14.80 μmol DPPH/ml wine with mean 5.96 μmol DPPH/ml (table 2). 'Chevalier de Rhodos' had the highest antiradical activity and 'methistanes' the lowest. Burnes et al. (2000) reported negative

relation between phenolic content and antiradical activity of several red wines. From the 16 red wine brands evaluated here ‘Chevalier de Rhodos’ had low total phenolic content (1630 ppm GAE) and high antiradical activity (14.80 μM DPPH/ml). ‘Grand Rose’ had greater total phenolic content (1430 ppm GAE) but five times lower antiradical activity (2.96 μM DPPH/ml) than ‘Chevalier de Rhodos’. ‘Rapsani’, ‘Peza’ and ‘Archodiko’ had intermediate content of total phenols and high antioxidant activity with 22.40, 20.02 and 22.45 μmol FRAP/ml respectively. The relation between total phenolic content and antiradical activity are shown in Figure 1.

Table 1.

Total Phenolic and anthocyan content of Greek Red Wines.

Wine Number	Wine Trade and Region	Variety	G.A.E. (ppm \pm sdev)	Catechin (μM)	Anthocyan (μm malv.)	Year, Grape category
n.1	Naoussa (Naoussa)	Ksinomavro	3805 \pm 75	13121	193 \pm 3	2005 V.Q.P.R.D.
n.2	Gumenissa (Gumenissa)	Ksinomavro + Negoska	3710 \pm 30	12793	193 \pm 3	2005 V.Q.P.R.D.
n.3	Ktima Averof (Ioannina)	Cabernet Sauvignon+ Cabernet Franc+ Merlot	3820 \pm 56	13172	209 \pm 7	2006 Regional
n.4	Rapsani (Larissa)	Ksinomavro + Krasato + Stavroto	2320 \pm 12	8000	428 \pm 8	2006 V.Q.P.R.D.
n.5	Ktima Katsaros (Larissa)	Cabernet Sauvignon + Merlot	2840 \pm 20	9793	208 \pm 9	2006 Regional
n.6	Messenikola (Karditsa)	Mesenikola + Syrah + Karinian	1420 \pm 45	4897	46 \pm 3	2004 V.Q.P.R.D.
n.7	Methistanes (Larissa)	Ksinomavro + Krasato + Stavroto	3970 \pm 68	13690	24 \pm 1	2004 Table grape
n.8	Sitia (Sitia)	Liatiko	3050 \pm 60	10517	10 \pm 1	2004 V.Q.P.R.D.
n.9	Peza-(Peza)	Kotsifali + Mandilari	2320 \pm 79	8000	29 \pm 3	2004 V.Q.P.R.D.
n.10	Daphnes (Daphne)	Liatiko	1360 \pm 22	4690	17 \pm 1	2004 V.Q.P.R.D.
n.11	Critikos (Crete)	Kotsifali + Mandilari	2740 \pm 11	9448	40 \pm 3	2005 Regional
n.12	Archodiko (Rodos)	Cabernet Sauvignon	2220 \pm 90	7655	65 \pm 2	2005 Regional

		+ Grenache Rouge				
n.13	Chevalier de Rhodos (Rodos)	Mandilaria	1630±31	5621	30±1	2005 V.Q.P.R.D.
n.14	Egeopelagitikos (Paros)	Monemvasia + Mandilaria	2880±31	9931	89±3	2006 Regional
n.15	Kalabaki (Limnos)	Limnio	2160±29	7448	76±2	2006 Table grape
n.16	Grand Rose (Rodos)	Amorgiano	1430±39	4931	20±1	2005 Table grape

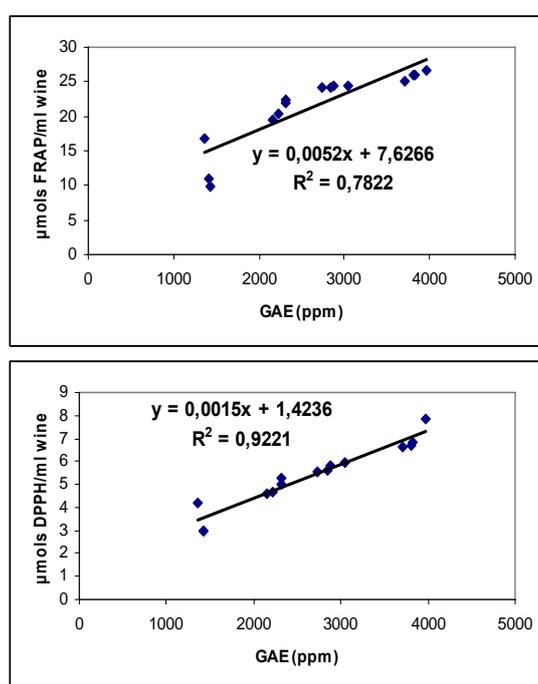


Figure 1. Relation between red wines antiradical and antioxidant activity and total phenolic content (GAE).

Table 2. Antiradical activity (DPPH) and Antioxidant activity (FRAP) of Greek Red Wines.

Wine Number	Trade	µM DPPH/ml	µM FRAP/ml	150 ml wine	
				µmol FRAP	eq. mg vit. C
n.1	Naoussa	6.70±0.10	26.00±0.10	3900	345
n.2	Goumenissa	6.64±0.20	25.11±0.22	3767	333
n.3	Ktima Averof	6.82±0.11	26.06±0.33	3909	346
n.4	Rapsani	5.28±0.25	22.40±0.10	3360	297

n.5	Ktima Katsaros	5.60±0.26	24.10±0.80	3615	320
n.6	Messenikola	2.96±0.12	10.90±0.32	1635	145
n.7	Methistanes	7.83±0.12	26.75±0.35	4013	355
n.8	Sitia	5.95±0.15	24.50±0.34	3675	325
n.9	Peza	4.98±0.15	22.02±0.50	3303	292
n.10	Daphnes	4.22±0.17	16.84±0.30	2526	223
n.11	Critikos	5.52±0.22	24.10±0.42	3615	320
n.12	Archodiko	4.70±0.17	20.45±0.40	3068	271
n.13	Chevalier de Rhodos	14.80±0.20	22.12±0.28	3318	294
n.14	Egeopelagitikos	5.85±0.28	24.40±0.88	3360	297
n.15	Kalabaki	4.58±0.11	19.48±0.35	2922	259
n.16	Grand Rose	2.96±0.04	9.95±0.10	1493	132

CONCLUSION

Greek red wines exhibit remarkable antioxidant and antiradical activity. Flavonoid and total phenol concentrations in Greek red wines provide a valuable tool for local nutraceutical industries, a key-role which will make them great competitors in the global market. High interest have the red wine brands 'Chevalier de Rhodos', 'Grand Rose' 'Rapsani', 'Peza' and 'Archodiko' as shown high relationship between total phenolic content and antioxidant or antiradical activity.

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