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TOTAL PHENOLS AND ANTIRADICAL ACTIVITY (DPPH) OF RED AND WHITE WINES FROM DIFFERENT REGIONS OF GREECE

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ABSTRACT

In the present research chemical analysis was made in 18 Greek wines, red and white wines, selected from different regions of Greece. The results showed that for all wines the total phenols (presented as Gallic acid equivalents or catechin) varies from 215 up to 3800 ppm. Antiradical activity for the red wines it was found higher with a mean value 4,64 μM, while the antiradical activity for the white wines it was found lower with a mean value 0,42 μM. The effective concentration (EC_{50}), concerning the phenols has a high value for all types of wines. Phenolic antioxidant index was higher for the red wines with values 153 up to 596 and lower for the white wines with values 14 up to 30. We concluded that phenolic antioxidant index it can be used as indicator of antioxidants force of wines.

INTRODUCTION

The grapes, the wine and the other products of grapes are object of many studies because are richly in naturally antioxidants (Halliwell, 1987; Kinsella et al., 1993; Kanner et al., 1994). Particularly rich in phenols, consequently and in antioxidants is the red wine compared to white wine (Frankel et al., 1993; Kinsella et al., 1993; Frankel et al., 1995; Kandaswami et al., 1993; Burda et al., 2001)

Concerning the above, in a laboratory study was examined and compared different methods, of estimate antioxidants and antiradical activity in Greek red and white wines, received from a different origin and produced in a different way (technology), using the methods described by De Gauljas et al., 1999; Fogliano et al., 1999; Simonetti et al., 1997 and Vinson et al., 1995.

MATERIALS AND METHODS

In this study we are included 18 trade Greek wines (both red and white wines). Those wines are from different regions in Greece (Epirus, Thessaly, Sterea Ellada, Attiki and Evia, Table 1) and are characteristics regions wines for the internal and external market.

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The total phenols were determined with the reagent of Folin-Ciocalteu as described by the method of Singleton and Rossi (1996) with the changes made by Badershneider et al., 1999. The method procedure includes the analysis of 20 μl of an adequately diluted wine added in 1580μl of distilled water, 100μl of the Folin- Ciocalteu and 360 μl of 20% Na_2CO_3. The extinction of this solution was measured at 750 nm on an UV-9200 spectrophotometer. Under the same conditions, a standard curve with gallic acid was recorded and the results were expressed in mg/l gallic acid (GAE).

The antiradical activity of each wine was assessed by the reduction in the content of the stable free radical 2,2-Diphenil-1-picrylhydrazil (DPPH), reacted with polyphenols in the wine, using the method of Brand-Williams. The mechanism of the reaction of phenol antioxidants with DPPH is well studied, the interaction proceeds stoichiometrically and the different colors of the two forms of DPPH and DPPH-H make possible the spectrophotometrical (colorimetric) determination. For this purpose, 50μl of adequately diluted wine were added to 2ml of methanol solution of DPPH with concentration of 6 10-5 mol/l at room temperature. The solution was well homogenized, and after 15 min the extinction of the solution at 515 nm on an UV-2000 unit was measured, Simultaneously, the E0 of a blank sample, containing distilled water instead of wine, was measured. The difference in the extinction values gives the amount of DPPH, which had reacted with polyphenols, and it was calculated using the Brand-Williams equation: $Abc_{515}=12509.(C_{DPPH})-2,58.10^{-5}$

The antiradical activity was expressed in mol DPPH scavenged by the phenol compounds in 1 ml of wine. The effective concentration of phenolic compounds was determined through DPPH and presented the total phenol amount reducing the initial free radical coloration by 50% (EC).

The phenolic antioxidant index (PAOXI) presented in this research as a ratio between the micromolar phenol concentration (as catechin) and EC_{50} for the same units as described by Vinson et al., 1995. The graphic relationship of cross-correlation between the quantity of total Phenols (TP) and antiradical activity (DPPH) were made in Excel. The statistical program Minitab ver. 12 was used for all statistical analysis.

RESULTS AND DISCUSSION

Results of the 18 tested wines, phenolic content are presented in Table 1. Those data (Table 1) shows a large variability of the total wine phenols (215-3800ppm GAE). That is in concept with other researchers concluded that the red wines are richer in total phenols compared to the white wines.

Our data (Table 1) confirmed that the red wines are richer in total phenols compared to white with a mean value of TP= 2212,5 ppm GAE. This result could be explained by a) the different technology used in production of red and white wine, b) the differences in the fermentation process and c) the duration of contact of wine with the seeds of grapes.

An increased content of total phenols in the red wines, from the three tested viniculture regions of Greece, are observed only in the wines from Epirous region made by the varieties Merlot,Cabernet Sauvignon, Cabernet Franc,Vlachiko and Bekari. While the Thessaly region wines, prepared by the varieties Messenikola, Syrah and Cabernet Sauvignon (n. 4, Messenikola) and (n. 6, Chatzimichali), are characterized in this research as wines with the lowest content of phenols (Table 1).

Concerning white wines, the variability of the total wine phenols are from 215 ppm up to 340 ppm (Table 1).

Table 1

Total Phenols for Red and White Wines.

Red wine number (n.)	Wine Trade and (*Region*)	G.A.E..	Katechini	Gallic acid μM	Year, Grape category
		ppm±sdev	μM		
Red Wines					
n.1	Ktima Averof Ioannina-(*Epirus*)	3800±66	13110	20212	2000 Regional
n.2	Kokkino Veloudo Ioannina-(*Epirus*)	3250±113	11213	17286	1999 Table grape
n.3	Rapsani Larissa-(*Thessaly*)	1850±28	6383	9840	1999 V.Q.P.R.D.
n.4	Messenikola Karditsa-(*Thessaly*)	1050±50	3623	5585	1999 V.Q.P.R.D.
n.5	Ktima Katsaros Larissa-(*Thessaly*)	2350±25	8108	12499	2000 Regional
n.6	Chatzimichali Atalanti-(*Sterea Ellada*)	1200±29	4140	6383	2000 Regional
n.7	Ktima Chatzimichali Atalanti-(*Sterea Ellada*)	2250±38	7763	11968	2000 Regional
n.8	Ktima Charlafti Attiki-(*Sterea Ellada*)	1950±29	6728	10372	2000 Regional
n.9	Panselinos-Rose -(*Evia*)	1150±29	3968	6117	2000 Table grape
White Wines					
n.10	Zitsa Ioannina-(*Epirus*)	280±15	966	1489	2001 V.Q.P.R.D.
n.11	Orion Ioannina-(*Epirus*)	280±9	966	1489	2000 Regional
n.12	Anchialos Volos-(*Thessaly*)	220±11	759	1170	1999 V.Q.P.R.D.
n.13	Tyrnavos, Larissa-(*Thessaly*)	320±8	1104	1702	2002 Regional
n.14	Nafsika, Anavyssos -(*Sterea Ellada*)	340±7	1173	1808	1999 Table grape
n.15	Megapanos Spata-(*Attiki*)	220±4	759	1170	2001 Regional
n.16	Filaretos (*Attiki*)	225±8	776	1197	2000 Table grape
n.17	Lac de Roche (*Sterea Ellada*)	215±4	742	1144	2001 Table grape
n.18	Ktima Chatzimichali Atalanti-(*Sterea Ellada*)	275±5	949	1463	2000 Regional

Those data presented a higher content of total phenols compared with the Italian and Spanish white wines. While similar in the content of total phenols with Greek white wines, tested in this research, are the white wines from French, Portugal and Hungary (Sato et al., 1996). The antiradical activity [DPPH] of the total phenols of the white wines, examined in this research, is presented in the table 2.

Table 2

Antiradical Activity and EC_{50} for Red and White Wines

Red wine number	Trade	Variety	μmolDPPH /ml	EC_{50} μM Kat.	PAOXI
Red Wines					
n.1	Ktima Averof	Merlot+Cab.Sauvignon + Cab. Franc	6,80±0,10	22	596
n.2	Kokkino Veloudo	Vlachiko + Bekari	5,79±0,10	35	320
n.3	Rapsani	Ksinomavro + Krasato + Stavroto	4,25±0,15	20	319
n.4	Messenikola	Messenikola + Syrah + Cab.Sauvignon	2,49±0,10	23	158
n.5	Ktima Katsaros	Cab. Sauvignon + Merlot	5,16±0,02	33	246
n.6	Chatzimichali	Cab. Sauvignon	2,42±0,10	18	230
n.7	Ktima Chatzimichali	Cab. Sauvignon + Syrah + Grenache Rouge	4,29±0,05	17	457
n.8	Ktima Charlafti	Cab. Sauvignon	3,95±0,02	14	480
n.9	Panselinos-Rose	Grenache Rouge	2,86±0,10	26	153
White Wines					
n.10	Zitsa	Debina	0,49±0,03	32	30
n.11	Orion	Chardoney + Debina	0,38±0,01	50	19
n.12	Anchialos	Roditis + Savatiano	0,83±0,03	55	14
n.13	Tyrnavos	Roditis	0,43±0,01	61	21
n.14	Nafsika	Savatiano + Assyrtiko	0,37±0,01	55	21
n.15	Megapanos	Savatiano	0,26±0,02	43	18
n.16	Filaretos	Savatiano	0,27±0,01	46	17
n.17	Lac de Roche	Savatiano	0,28±0,01	42	18
n.18	Ktima Chatzimichali	Robola	0,48±0,01	38	25

From this (Table 2) it appears that the red wines have higher antiradical activity compared with the white wines, with a mean of 4,39 μmolsDPPH/ml and 0,42 μmols DPPH/ml for the red and the white wines respectively. Overall, in this research, the red wines from the region of Epirus (Ktima Averof, n.1 and Kokkino Veloudo) are wines characterized with highest antiradical activity with 6,80 and 5,79 μmols values respectively (Table 2). Interest presents the red wine (Ktima Katsapos, n.5) that has medium content total phenols 2350 ppm and high antiradical activity 5,16 μmols DPPH. In the white wines the antiradical activity shows a variation from 0,26μmols up to 0,83μmols.

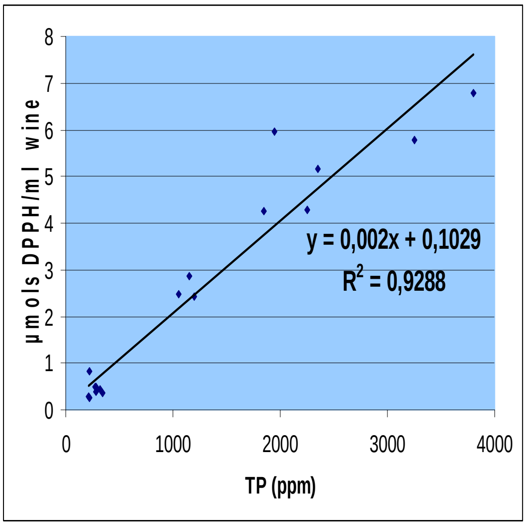

Figure 1. Correlation of Antiradical Activity and Total Phenols (GAE) in the tested wines.

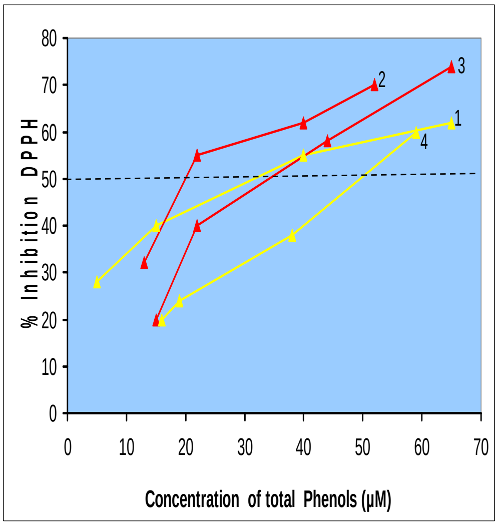

Figure 2. EC_{50} in wines from Epirus
1. Zitsa, EC_{50}=32;
2. Ktima Averof, EC_{50}=22;
3. Kokkino Veloudo, EC_{50}=35;
4. Orion, EC_{50}=50.

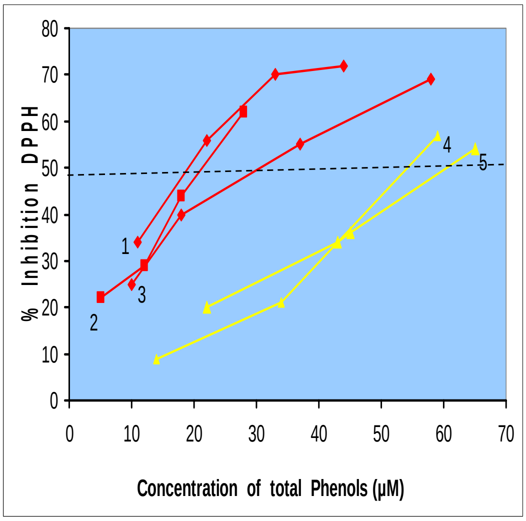

Figure 3. EC_{50} in wines from Thessaly
1. Rapsani, EC_{50}=20;
2. Messenikolas, EC_{50}=23;
3. Ktima Katsarou, EC_{50}=33;
4. Tyrnavos, EC_{50}=61;
5. Anchialos, EC_{50}=55.

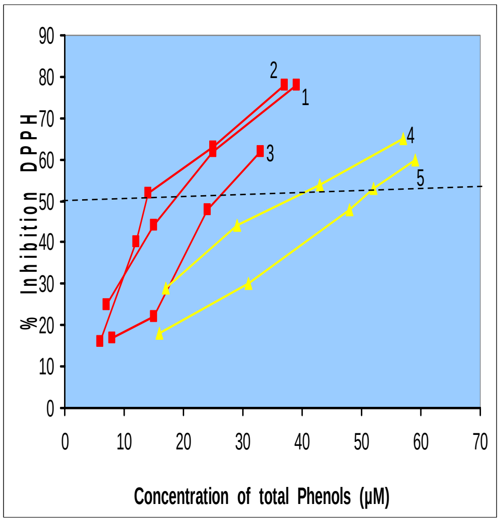

Figure 4. EC_{50} in wines from Sterea Ellada.
1. Chatzimichali, EC_{50}=18;
2. Ktima Chatzimichali, EC_{50}=17;
3. Panselinos, EC_{50}=26;
4. Ktima Chatzimichali, EC_{50}=38;
5. Filaretos, EC_{50}=46.

Generally, in wines is observed a very high value of cross-correlation between antiradical activity and TP with regression equation Y_{DPPH}=0,002.X_{TP}+0,1029 and r^2 =0,9288 (Sato et al., 1996; Frankel et al, 1995; Simonetti et al, 1997)

These researchers but also others agree that for the estimate of the wine antiradical activity, the wine EC_{50} (Fig. 2, 3 and 4) should be known. The red wines have an average EC_{50} =23 and the white an average EC_{50}=47. But in totals red wines are characterized with low EC_{50} values in other words high PAOXI, as the wines presented in this research with numbers n.1, n.3, n.4, n.6, n.7 and n.8 (Table 2). This resulted positive for the contents of free radicals of those wines, same data where presented by Kinssela et al., Hurtado et al., 1997; 1993; Leighton et al., 1998 and Nutall et al., 1999.

In this study only a small number from the white wines have a low EC_{50} value compared to the red wines e.g. the red wine Zitsa (n. 10 in this research).

Overall, from the results presented in this study we can conclude that wine's antioxidant and therapeutic action depends not only from the total content of phenols but also from the composition of individual wine total phenols and of course the climatic and geographic environment factors.

REFERENCES

1. Badershneider B, D. et al.1999, Vitis,38(3),127-131.
2. Brand Williams W, et al. 1995 .Lebensm.-Wiss.u,-Technol, 28,25-30.
3. Burda S,et al 2001, J. Agric. Food Chem., 49, 2774-2779.
4. Burns G, et al, 2000, J. Agric. Food Chem., 48, 220-230.
5. De Gaulejac S., et al, 1999. J. Agric. Food Chem. 47, 425-431.
6. Fogliano V, et al, 1999, J. Agric. Food Chem. 47, 1035-1040.
7. Frankel E, et al, 1995, J. Agric. Food Chem.43, 890-894.
8. Halliwell B., et al, 1989, Free Radicals in Biology and Medicine, 2nd ed., Oxford, U.K.
9. Hurtago I., et al, 1997, J. Agric. Food Chem., 45, 1283-1289.
10. Kandaswami C. et al, 1993, In Natural Antioxidants
11. Kanner J., et al 1994, J. Agric. Food Chem., 42, 64-69.
12. Kinssela E. J, et al, 1993, Food Technology, 85-89, April,
13. Leighton F, 1998. Bull. d, O.I.V., 807-808.
14. Mc Danal M.S., et al, 1998, J. Agric. Food Chem., 46, 368-375.
15. Nutall S. L., et al., 1999, O.S.M. 92, 239-244.
16. Robak J., 1988, Biochem. Pharmaced., 37 (5) 837-841.
17. Sato M. N., et al., 1996, J. Agric. Food Chem., 44, 37-41.
18. Satue-Gracia M. C., et al., 1999, J. Agric. Food Chem., 47, 2198-2202.
19. Singleton V. L., et al., 1965, J. Enol. Vitic., 16, 144-158.
20. Simonetti P., et al., 1997, J. Agric. Food Chem., 45, 1152-1153.
21. Yen G-C, et al., 1995, J. Agric. Food Chem., 43(1), 27-32.
22. Vinson J. A., et al., 1995, J. Agric. Food Chem.