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PHENOL CONTENT AND ANTIOXIDANT ACTIVITY OF SOME BULGARIAN AND GREEK WINES

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Abstract. The total phenol content of 25 different red, rose, and white wines tested ranged from 260 to 3950 ppm. Their antiradical activities were from 0.38 to 7.44 μmol DPPH•/ml and depended on the respective phenolic contents (r^2 = 0.8036). The high quality (OPAP) red wines from Nausa, Amanteo, and Gumenitza were characterised by low anthocyanin contents and EC_{50} and a high antioxidant index (PAOXI). The antioxidant activity depended strongly on the total phenol content (r^2 = 0.9494) and ranged from 1.30 to 30.72 μmol FRAP/ml. The deviations from the direct correlation between phenolic content, antiradical- and antioxidant activities of wines were explained by the respective cultivar and the specific ecological conditions of grape growing, which affected the group- and individual composition of phenolic compounds.

Keywords: wine, phenols, antioxidants, DPPH•, FRAP.

AIMS AND BACKGROUND

In recent years there has been increasing interest by food industry and preventive medicine in 'natural antioxidants' of plant origin.

Plants synthesise a large number of secondary substances some of which, such as tocopherols, ascorbic acid and carotenoids, are antioxidants. Phenolic compounds and flavonoids, in particular, are widespread in plants and are involved in their protection against oxidative and other damages[1]. Oxidative damages are caused by the so-called reactive oxygen species (ROS) formed in all aerobic organisms. In man, they cause the occurrence of many diseases including cardio-vascular, atherosclerotic, cancerogenic, etc.[2]

The phenols found in grapes and wine exerted different biological effects, including antioxidant ones[3].

The so-called 'French paradox' (a reduced mortality from cardio-vascular diseases in spite of saturated fat consumption) was explained by wine consumption[4]. Many investigations emphasised the association between the phenol content of wines and their antioxidant and therapeutic effects on the one hand, and the correlation of these substances with the environmental conditions of grapevine growing and the technological conditions of wine making, on the other[5-8].

Bulgaria and Greece are traditional producers of grapes and wine. Some of grape cultivars and their wines are typical for both countries. It is of interest to study the phenol content and antioxidant effects of some wines produced from cultivars grown under similar ecological conditions in South Bulgaria and North Greece.

EXPERIMENTAL

The study involved 25 brands including, red, rose and white wines. Amongst the Bulgarian wines there were selected such produced from the South Bulgarian Plovdiv region – the producer of the traditional wine of grape cv. Mavrud. The Greek wines were represented mainly by the so-called OPAP wines (V.Q.P.R.D.) – high quality wines from controlled regions, such as Nausa, Aminteo and Gumenitza, produced mainly from cv. Xinomavro (Table 1).

Table 1. Wines tested

No	Brand, colour	Producer	Grape cultivar	Region
1	2	3	4	5
		South Bulgaria – regionalised wines		
1	Mavrud (red)	AU-Plovdiv	Mavrud	v. Brestnik
2	Mavrud (red)	Pazardzik	Mavrud	v. Karabunar
3	Mavrud (red)	Vinprom-Assenovgrad	Mavrud	town of Assenovgrad
4	Mavrud (red)	v. Brestovitza	Mavrud	v. Brestovitza
5	Cabernet (red)	town of Stamboliiski	Cabernet	town of Stamboliiski
6	Evmolpia (red)	AU-Plovdiv	Evmolpia	v. Brestnik
7	Chardonnay (white)	AU-Plovdiv	Chardonnay	v. Brestnik
		North Greece – high quality wines V.Q.P.R.D. (OPAP)		
8	Nausa (red)	Butari	Xinomavro	Nausa
9	Xinomavro (red)	Butari	Xinomavro	Nausa (Stanimaka)
10	Aminteo (red)	Koop	Xinomavro	Aminteo
11	Gumenitza (red)	Butari	+ Negoska	Gumenitza
12	Limnio (red)	Porto Karas	Limnio + Xinomavro	Melitona
13	Aminteo (rose)	Koop	Xinomavro	Aminteo
14	Porto Karas (white)	Porto Karas	Assirtiko + Atiri + Roditis	Melitona
		Local wines (TOPICO)		
15	Merlot (red)	Zantali	Merlot	Chalkidiki
16	Cabernet Sauvignon (red)	Protopapas	Cabernet	Pageortikos
17	Gianakahori (red)	Butaris	Xinomavro	Imatias
18	Xinomavro + Merlot (red)	Ligas	Xinomavro + Merlot	Pelas

to be continued

Continuation of Table 1

1	2	3	4	5
19	wine-cellar Ligas (red)	Ligas	Merlot	Pelas
20	Roditis (white)	Zantali	Roditis	Macedonia
21	Orinos (white)	Chalkidiki	Roditis	Florina
22	Roditis (white)	Ligas	Roditis	Pelas
23	Macedonikos (rose)	Butaris	Xinomavro	Macedonikos
24	Domen Karas (rose)	Porto Karas	C. Sauvignon + Limnio + Cab. fran	Chalkidiki
		Traditional wines		
25	Malamatina (Retzina)	Malamatinas	Savatjano + Limnio	Thessalonike

Note: All wines were produced in the year 2000, except No 4 (1999), No 12 (1999), No 18 (2001), No 25 (2002).

The total phenols were determined with the reagent of Folin-Ciocalteu by the method of Singleton and Rossi[9] and its microvariant[10].

The antiradical activity was assessed by the stable free radical 2,2′-diphenyl-1-picrylhydrazyl (DPPH•) using the method of Brand-Williams et al.[11] The effective concentration of phenolic compounds was determined through DPPH· and presented the total phenol amount reducing the initial free radical colouration by 50% (EC_{50}). The phenolic antioxidant index (PAOXI) presented the ratio between the micromolar phenol concentration (as catechin) and EC_{50} in the same units[6].

The antioxidant activity of wines was assessed using the FRAP method (ferric reducing/antioxidant power) of Benzie and Strain[12]. It was expressed as μmol FRAP/ml wine. The antioxidant activity of the L-ascorbic acid standard was 11 304 μmol/g.

RESULTS AND DISCUSSION

The total phenol content in the Bulgarian wines tested ranged from 260 to 3200 ppm, and that in the Greek ones – from 225 to 3950 ppm (Table 2). The wines produced from the traditional grape cultivar Mavrud contained from 2300 to 2850 ppm, and those from the traditional cv. Xinomavro – from 3100 to 3850 ppm phenols. The two rose regionalised wines (TOPICO) (Nos 23 and 24), produced from cv. Xinomavro, even if by different producers, had close phenol contents – 770 and 850 ppm, respectively. The highest phenol content was found in the regionalised wine (No 18) produced in the Ligas wine-cellar from cvs Xinomavro + Merlot – 3950 ppm.

Table 2. Total phenols and anthocyans wines

No	Wines tested, regions	Gallic acid		Catechin	Anthocyans
		(ppm)	(µM)	(µM)	(µM malv.)
	South Bulgaria – regionalised wines				
1	Mavrud – v. Brestnik	2850	15160	9828	265
2	Mavrud – v. Karabunar	2500	13298	8621	56
3	Mavrud – town of Assenovgrad	2800	14893	9655	74
4	Mavrud – v. Brestovitza	2300	12234	7931	
5	Cabernet – town of Stamboliiski	3200	17021	11034	274
6	Evmolpia – v. Brestnik	2000	10638	6897	282
7	Chardonnay white – v. Brestnik	260	1383	897	–
	North Greece – high quality V.Q.P.R.D. (OPAP)				
8	Nausa – Nausa	3100	16489	10690	
9	Xinomavro – Nausa	3700	19680	12759	78
10	Aminteo – Aminteo	3750	19947	12793	36
11	Gumenitza – Gumenitza	2600	13830	8966	37
12	Limnio – Melitona Hills	2550	13564	8793	27
13	Aminteo (rose) – Aminteo	770	4095	2655	
14	Porto Karas (white) – Melitona Hills	500	2660	1724	–
	Regionalised wines (TOPICO)				
15	Merlot – Chalkidiki (red)	1800	9574	6207	
16	Cabernet – Pateortikos (red)	1850	9840	6379	160
17	Gianakaheri – Imatias (red)	3850	20479	13276	138
18	Xinomavro + Merlot – Pelas (red)	3950	21011	13621	204
19	wine-cellar Ligas – Pelas (red)	3850	20479	13276	27
20	Roditis (white) – Macedonia	225	1197	779	–
21	Orinos (white) – Florina	300	1596	1034	–
22	Roditis (white) – Pelas	380	2021	1310	–
23	Macedonikos (rose) – Macedonia	850	4521	2930	7.5
24	Domen Karas – Chalkidiki (rose)	750	3980	2587	11
	Traditional wines				
25	Malamatina – Retzina, Thessalonike	270	1436	930	–

The white wines of North Greece, produced from cv. Roditis, were characterised by an average phenol content of 294 ppm, and the Bulgarian Chardonnay – by 260 ppm. An exception was the wine Porto Karas (No 14), produced from the three cvs Assirtiko, Atiri and Roditis, containing 500 ppm phenols.

The anthocyan content in the evaluated red wines varied in wide ranges – from 27 to 280 µM malvidin-3-glucoside. The high-quality (OPAP) wines from North Greece had a low anthocyan content, while some of the regionalised

(TOPICO) and Bulgarian regionalised wines contained both more (Nos 1, 5, 6, 16, 17 and 18) and less (Nos 2, 3 and 19) anthocyans.

The results for the antiradical activity of the wines tested, measured by the inhibition of the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH•), the effective concentration (EC_{50}) and the phenolic antioxidant index (PAOXI) are shown in Table 3.

Table 3. Antiradical activity (DPPH•)

No	Wines tested, regions	µmol DPPH•/ ml wine	EC_{50} ($\mu M_{cat.}$)	ARP ($\mu M_{cat.}$)	PAOXI
	South Bulgaria – regionalised wines				
1	Mavrud – v. Brestnik	5.12	35	0.0286	281
2	Mavrud – v. Karabunar	4.96	28	0.0357	314
3	Mavrud – town of Assenovgrad	5.12	50	0.0200	193
4	Mavrud – v. Brestovitza	3.78	29	0.0345	130
5	Cabernet – town of Stamboliiski	6.62	27	0.0370	401
6	Evmolpia – v. Brestnik	5.52	30	0.0333	300
7	Chardonnay(white) – v. Brestnik	0.44	39	0.0256	23
	North Greece – high quality V.Q.P.R.D. (OPAP)				
8	Nausa – Nausa	6.50	36	0.0277	297
9	Xinomavro – Nausa	7.44			
10	Aminteo – Aminteo	7.41			
11	Gumenitza – Gumenitza	5.50	38	0.0263	236
12	Limnio – Melitona Hills	5.06	37	0.0270	236
13	Aminteo (rose) – Aminteo	2.50	77	0.0130	34
14	Porto Karas (white) – Melitona Hills	1.63	45	0.0222	38
	Regionalised wines (TOPICO)				
15	Merlot – Chalkidiki	3.90	34	0.0294	183
16	Cabernet – Pageortikops	3.86	38	0.0263	170
17	Gianakahori – Imatias	6.51	39	0.0256	345
18	Xinomavro + Merlot – Pelas	6.86	14	0.0714	973
19	wine-cellar Ligas (Merlot) – Pelas	6.85	22	0.0455	603
20	Roditis (white) – Macedonia	0.62	–	–	–
21	Orinos (white) – Florina	0.47	–	–	–
22	Roditis (white) – Pelas	0.34	88	0.0119	15
23	Macedonikos (rose)	2.53	23	0.0435	130
24	Domen Karas (rose)	2.54	21	0.0476	123
	Traditional wines				
25	Malamatina Retzina, Thessalonike	0.38	48	0.0208	19.39

The highest antiradical activity was typical for the red wines tested – 6.12 µmol DPPH•/ml on the average, followed by the rose ones with 2.52 µmol, and the white wines with 0.47 µmol.

The highest antiradical activity was found in the high-quality (OPAP) wines from Nausa and Aminteo (Nos 8, 9 and 10), and the regionalised (TOPICO) wines from Imatias and Pelas (Nos 17-19), as well as in the Cabernet from the town of Stamboliiski (No 5). The antiradical activity shown by the white wine Porto Karas (No 14) was almost four times higher than that in the other white wines tested. Most of the wines from South Bulgaria and North Greece had similar effective concentrations (EC), i.e. close antiradical powers (ARP) of their phenolic compounds. The highest antiradical power was established in the polyphenols of the red wines from Pelas (Nos 18, 19) and the rose Macedonikos and Domen Karas (Nos 23, 24), while the lowest one was found in the white wine from Pelas (No 22) and the rose one from Aminteo (No 13).

Table 4 shows that the antioxidant activity, determined by the FRAP method of Benzie and Strain (1999) was highest in the red wines – 22.10 μmol FRAP/ml wine, followed by the rose – 7.91 μmol and the white wines – 2.34 μmol FRAP/ml wine. The South Bulgarian red wines tested were characterised by a lower ferric reducing activity as compared to the high-quality (OPAP) red wines and some regionalised red wines (TOPICO) from North Greece. The highest activity was established in the phenolic substances of the Imatias wine (No 17) and the Xinomavro + Merlot wine from Pelas (No 18). There was a high correlation (r^2 = 0.9494) between the phenolic content of wines and their ferric reducing activity, some exceptions being also observed, such as the case with the Merlot wine (No 19) from the Ligas cellar.

The average total phenol content, determined with the reagent of Folin-Ciocalteu, was 2992 ppm for the red wines, 790 ppm for the rose ones and 322 ppm for the white wines, confirming the results obtained previously by other authors[5-8,13-15]. These authors suggested that the phenolic content of wines was mainly affected by the specific technologies of red, rose and white wine-making, as well as by the grape cultivar and the different ecological factors of grape production. The results for the total phenols in wines produced from the same cultivar (e.g. Mavrud or Xinomavro), by standard technologies, showed that they depend on the specific regional conditions of grape growing (soil composition, climate, altitude, solar radiation, etc.). In North Greece, such specific regions for cv. Xinomavro are Nausa, Aminteo, Gumenitza, Imatias, and Pelas, and in South Bulgaria for cv. Mavrud – Assenovgrad, Brestovitza, Stamboliiski, and Brestnik.

Many authors determined the antiradical activity of phenolic compounds by inhibition of the stable free radical DPPH• (Refs 15-19).

In our studies, the antiradical activity of the wines tested was expressed in μmols inhibited DPPH• according to the method of Brand-Williams et al.[11] A high correlation was established between the total phenol content and the antiradical activity of the wines tested (r^2 = 0.8036). Some deviations from this correlation were observed in wines Nos 6, 7 and 19, which could be explained by the

Table 4. Antioxidant activity (ERAP)

No	Wines tested, regions	μmol FRAP / ml wine	150 ml wine	
			μmol FRAP	eq. mg vit. 'C'
	South Bulgaria – regionalised wines			
1	Mavrud – v. Brestnik	18.16	2724	242
2	Mavrud – v. Karabunar	14.72	2208	194
3	Mavrud – town of Assenovgrad	17.84	2676	235
4	Mavrud – v. Brestovitza	15.48	2322	204
5	Cabernet- town of Stamboliiski	20.96	3144	277
6	Evmolpia – v. Brestnik	16.64	2496	220
7	Chardonnay (white) – v. Brestnik	2.12	318	28
	North Greece – high quality V.Q.P.R.D. (OPAP)			
8	Nausa – Nausa	23.50	3525	310
9	Xinomavro – Nausa	27.16	4074	358
10	Aminteo – Aminteo	26.60	3990	351
11	Gumenitza – Gumenitza	23.81	3572	288
12	Limnio – Melitona Hills	22.30	3345	294
13	Aminteo (rose) – Aminteo	9.07	1361	120
14	Porto Karas (white) – Melitona Hills	5.32	798	70
	Regionalised wines (TOPICO)			
15	Merlot – Chalkidiki	15.90	2385	210
16	Cabernet- Pageortikos	15.20	2280	201
17	Gianakahori – Imatias	30.10	4515	397
18	Xinomavro + Merlot – Pelas	30.72	4608	405
19	wine-cellar Ligas (Merlot) – Pelas	25.70	3855	339
20	Roditis (white) – Macedonia	1.30	190	17
21	Orinos (white) – Florina	1.50	225	20
22	Roditis (white) – Pelas	1.72	258	23
23	Macedonikos rose	7.31	1097	96
24	Domen Karas rose	7.34	1101	97
	Traditional wines			
25	Malamatina Retzina, Thessalonike	2.09	314	28

different ratios of the specific phenolic groups and their individual forms[8,14-16,20]. The antioxidant activity expressed as a ferric reducing power of the complex Fe^{3+}-tripyridyltriazine, was determined using the method of Benzie and Strain[12] as well as in a correlative relationship with the total phenols (r^2 = 0.9494). A similar relationship between the total-, group- or individual phenols in wine were also established by other authors[14,15,21,22]. In our previous study of 30 Bulgarian and Greek wines we established a high correlation coefficient between the total phenols and their ferric reducing power – r^2 = 0.9208. A deviation from this correlation was observed in some regionalised (TOPICO) wines from North

Greece and South Bulgaria (Nos 4, 15, 16 and 19). The highest ferric reducing activity was found in wines Nos 17 and 18, as well as in the OPAP wines from Nausa and Aminteo. The activity exhibited by the white wine Porto Karas was more than three times higher than that of the other white wines.

The deviations from the correlation between the total phenols and their ferric reducing activities were probably due not only to differences in the individual composition of phenolic compounds in wine, but also to their synergic effect.

CONCLUSIONS

The wines from South Bulgaria and North Greece, produced in ecologically sound regions, manifested high antioxidant (FRAP) and antiradical ($DPPH^{\bullet}$) activities. The higher antioxidant properties of red wines were associated with their high total phenol content and the specific individual phenols (EC_{50}), which also determined the differences in the total polyphenolic antioxidant index (PAOXI). The antioxidant and antiradical properties of wines depended on the specific cultivar, the ecological region of grape growing, and the wine-making technology used.

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