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Principal phenolic compounds in Greek red wines

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Abstract

Polyphenolic substances are of profound significance to both the technological and nutritional value of grapes (*Vitis vinifera* sp.) and wines. The Hellenic vineyard embraces a large number of native cultivars, with various phenolic contents, which are mostly unexploited. For this reason, 20 red wines, including nine made by rare native Greek varieties, were assayed for their polyphenolic content, using high performance liquid chromatography coupled with a diode array detector. All wines examined were produced under identical enological practices within the premises of the Wine Institute of Athens, so that the observed differences were related to grape variety and geographical origin (in some cases). The results showed that some of the unexploited rare native varieties (e.g., Karvouniaris, Thrapsa, Nerostafilo, Bakouri, Vertzami) contained appreciable amounts of non-colored phenols as well as anthocyanins meaning that they would be worthy of further study and use for the production of quality wines. Karvouniaris, Thrapsa and Augoustiatis were found high in phenolic acids and flavanols but low in flavonols and hydroxycinnamic acids. Nerostafilo and Bakouri were also rich in phenolic acids and flavanols but the former was poor in flavanols and the latter poor in hydroxycinnamates. Finally, by applying PCA to the results some grouping was observed.

Keywords: Greek wines; Polyphenols; Anthocyanins; Autochthonous cultivars

1. Introduction

Currently, much attention has been devoted to nutritional antioxidants due to their association with suppressed rates of degenerative diseases such as cardiovascular disorders (Knekt, Jarvinen, Reunanen, & Maatela, 1996) and cancer (Kato, Nakadate, Yamamoto, & Sigimura, 1983; Verma, Johnson, Gould, & Tanner, 1988). There is accumulating evidence that plant phenolic compounds inhibit oxidation of low-density lipoprotein (Frankel, Kanner, German, & Kinsella, 1993) both in vitro and in vivo and reduce platelet aggregation. Red wine is an excellent source of various classes of polyphenols and may contain 1000–4000 mg l^{-1} of flavonoids which have different biological activities (Arnous, Makris, & Kefalas, 2001). Flavonoids consist mainly of anthocyanins, flavonols, flavones, isoflavones and flavanols.

It should be noted, however, that grape phenolic compounds besides their antioxidant properties are very important constituents of wines since they contribute to color, astringency and bitterness (Robichaud & Noble, 1990), oxidation reactions (Cheynier & Ricardo Da Silva, 1991; Ozmianski & Sapis, 1989), interactions with proteins (Mehanso, Butler, & Carlson, 1987; Ricardo Da Silva et al., 1991) and ageing behavior of wines (Haslam, 1980).

Viticulture and wine making in Greece have been widely practiced since antiquity. Continuous and intensive selection of grape varieties that favoured the production of desired wine styles led over the centuries to a plethora of native cultivars (*Vitis vinifera* sp.), which possess various distinct enological characteristics and organoleptic properties. However, today, most of these native varieties are becoming rare since they are replaced by the well-known French ones (e.g., Cabernet Sauvignon, Merlot, Syrah) due to their repute in producing quality wines.

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Because of their unique varietal (genetic) diversity, Greek grapes may vary largely in the composition of certain specific constituents, which define decisively the overall quality of wines. Therefore, the examination of wine composition is an indispensable element in choosing (and/or blending) the appropriate grape varieties and selecting the technological applications which enable the production of high quality wines.

One of the major parameters that is of both technological and nutritional significance is the flavonoid composition. Particularly for the grape varieties cultivated in Greece and the wines produced, the polyphenolic composition has not been examined in detail, although some investigations have been carried out on native red cultivars (Lanaridis & Bena-Tzourou, 1997) and wines (Arnous, Makris, & Kefalas, 2002a; Arnous, Makris, & Kefalas, 2002b; Dourtoglou, Yannovits, Tychopoulos, & Mamvakias, 1994; Kallithraka, Arvanitoyannis, El-Zajouli, & Kefalas, 2001; Kallithraka et al., 2001; Sakkiadi, Haroutounian, & Stavrakakis, 2001; Verma et al., 1988). However, all the above investigations were focused on commercial Greek varieties used mainly to produce wines of appellation of origin. There exist a number of cultivars which were not exploited for their potential to produce quality wines and therefore ignored by the wine producers.

Therefore, it was thought that determining the flavonoid composition of several, red wines produced by rare native varieties (*Vitis vinifera* sp.), would be of great importance in obtaining a general picture of their potential for commercial use. For that purpose, the national collection of the ''Vine Institute of Athens'' was used in order to provide the grapes for the experimental wines which where produced under identical enological practices within the premises of the Wine Institute of Athens. In this way, the variation due to environmental factors (soil type and climate) and enological techniques were eliminated. In addition, it was interesting to obtain a comparison between them and selected appellation of origin wines made by local varieties grown in the appropriate geographical parts of Greece. However, in order to have comparable results, the wines were produced in the same winery and under similar technological conditions. Similarly, two wines made by the widely used French varieties (Cabernet Sauvignon, Syrah) were studied for comparison reasons.

2. Materials and methods

2.1. Wines

All varieties used were *V. vinifera* species. Twelve varieties were chosen from the collection of the Vine Institute (Likovrisi, Athens), six of the ''appellation of origin'' geographical areas of Greece and two of the so-called ''international'' cultivars, which are cultivated in Greece. Details about the cultivars and their location are given in Table 1. The grapes used for the production of the experimental wines were harvested at optimum technological maturity, as judged by indices of sugar and acid content, established by the Institute of Wine. All the wines tested were produced in the winery of the Wine Institute under similar oenological practices and stored under similar conditions. Crushed grapes stayed in contact with the must for six days at 15–18 °C. All samples were analysed between February and March in the year following that of vintage (2002).

2.2. HPLC determination of anthocyanins

Wine samples were filtered through 0.45 μm syringe filters prior to high pressure liquid chromatography (HPLC)

Table 1
Variety and origin of the wines tested

Wine codes	Cultivar	Location
1	Karvouniaris	National Collection, Vine Institute, Athens
2	Thrapsa	National Collection, Vine Institute, Athens
3	Nerostafilo	National Collection, Vine Institute, Athens
4	Bakouri	National Collection, Vine Institute, Athens
5	Kotselina	National Collection, Vine Institute, Athens
6	Limniona	National Collection, Vine Institute, Athens
7	Viodomatis	National Collection, Vine Institute, Athens
8	Augoustiatis	National Collection, Vine Institute, Athens
9	Moshato Amvourgou	National Collection, Vine Institute, Athens
10	Vertzami	National Collection, Vine Institute, Athens
11	Barbera	National Collection, Vine Institute, Athens
12	Refosko	National Collection, Vine Institute, Athens
13	Syrah	Central Greece
14	Cabernet Souvignon	Central Greece
15	Krasato	Central Greece (Rapsani)
16	Stavroto	Central Greece (Rapsani)
17	Mavro Mesenikola	North Greece (Karditsa)
18	Agiorgitiko	South Greece (Peloponnese)
19	Xinomavro	North Greece (Goumenissa)
20	Mandilaria	Aegean islands (Paros)

analysis. The equipment consisted of a HP 1050 chromatography apparatus coupled to a diode array detector. Analyses were performed, as in Kallithraka, Mohdaly, Makris, and Kefalas (2005), on a Spherisorb ODS-2 column 5 μm, 250 × 4 mm, at a flow rate of 1 ml min^{-1}, using a 20 μl injection volume, detection at 520 nm, and the following elution programme: 95% eluent A for 1 min, then from 95% to 50% in 25 min, from 50% to 5% in 3 min, which was kept isocratic for a further 3 min. Eluent A was 10% aqueous formic acid and eluent B MeOH. Identification was based on comparing retention times of the peaks detected with those of original compounds, and on UV–vis on-line spectral data. Malvidin-3-*O*-glucose coumarate (MvCoum) was tentatively identified based on previous observations (Arnous et al., 2002a). Quantification was accomplished using the standard anthocyanin solution, while MvCoum was quantified as Malvidin-3-*O*-glucose (Mv). Results were expressed as mg l^{-1}. All analyses were performed in triplicate.

2.3. HPLC determination of individual phenolics

The concentration of individual polyphenols was determined by HPLC, according to the method described by Kallithraka et al. (2001). A Hewlett-Packard 1050M Series II with an auto injector (25 μl injection volume) and a diode array detector, recording at 280, 320, 365 and 265 nm, was used to detect the phenolic compounds. A reversed phase ODS-2 Shperisorb column (250 × 4 mm i.d., particle size 5 μm) at 40 °C was used with a flow rate of 1 ml min^{-1}. Using 0.6% aqueous perchloric acid and methanol as eluents, the following linear gradient was used: in 55 min from 5% to 80% methanol, hold for 15 min at 80% methanol to wash the column and then return to the initial conditions to re-equilibrate for 10 min.

Peaks were identified by comparison of retention times and ultraviolet (UV) spectra with commercial standards: gallic acid, protocatechuic acid, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, (+)-catechin, (−)-epicatechin, myricetin, quercetin, kaempherol, rutin (all from Sigma), and procyanidins B1, B2, C1 and A2 (gift from Dr. A.G.H. Lea, Reading, UK). Procyanidins are expressed as mg l^{-1} (+)-catechin, whereas the rest of the compounds are expressed against their own calibration curves. All analyses were performed in duplicate.

2.4. Total phenols

The total phenols were determined using the Folin–Ciocalteau method (Waterman & Mole, 1994) and are expressed as mg l^{-1} gallic acid (GAE). All analyses were performed in triplicate.

2.5. Statistical analysis

In all cases analyses were performed in triplicate, unless elsewhere specified, and the values were averaged. The standard deviation (SD) was also calculated. In addition, Principal Component Analysis (PCA) was carried out with SPSS base 9.0 (SPSS, 1999).

3. Results and discussion

3.1. Total phenols and total anthocyanins

The results related to the determination of phenolic compounds and anthocyanins in Greek wines are summarized as mean values and standard deviations in Tables 2–7.

It should be mentioned that the phenolic content of the wines made from the following native rare grape varieties: Karvouniaris, Thrapsa, Nerostafilo, Bakouri, Kotselina, Limniona, Voidomatis and Augoustiatis (wine codes 1–8, respectively, in Table 1) has never been analysed in the past. In addition, wines made from Krasato, Stavroto and Mavro Mesenikola (wine codes 15–17, respectively) have not been analysed separately in the past. Only mixtures of the above mentioned varieties have been analysed by Kallithraka et al. (2001). Furthermore, it is the first time that wines made from the Italian varieties Barbera and Refosko (wine codes 11 and 12, respectively) grown in Greece have been analysed.

Total phenolic content of the wines examined (Table 2) varied from 622 (wine No. 17) to 3200 (wine No. 2), the average being 2102 GAE. These results fell within the range

Table 2
Total phenolic[a] (TP) and total anthocyanin[b] (TA) content of the wines examined

Wine codes	TP	TA
1	2752.4 ± 13.5	194.23
2	3200.3 ± 18.3	352.23
3	2360.7 ± 12.2	312.03
4	2422.2 ± 10.4	350.42
5	2199.4 ± 19.4	18.59
6	2065.3 ± 18.7	463.11
7	2271.1 ± 17.1	21.00
8	2377.9 ± 12.2	496.47
9	1822.7 ± 18.7	57.91
10	2469.2 ± 16.9	1011.83
11	960.2 ± 15.8	212.47
12	828.5 ± 14.9	324.88
13	1919.5 ± 18.7	457.92
14	2481.0 ± 10.4	698.91
15	708.4 ± 15.5	20.15
16	2373.7 ± 13.6	104.86
17	621.7 ± 16.0	22.78
18	2283.0 ± 16.8	402.7
19	3000.2 ± 10.6	160.82
20	2917.7 ± 19.8	478.41
Average	2101.7	308.1

Wine codes are as in Table 1.

[a] Values represent means of triplicate determinations ($n = 3$) ±SD. Data are expressed as gallic acid equivalents (mg l^{-1}).

[b] Values are the summary of the concentrations determined by HPLC (mg l^{-1}).

Table 3
Analytical phenolic acid composition of the wines tested[a]

Wine codes	Gallic acid	Protocatechuic acid	Syringic acid	Vanillic acid	Total
1	135.20 ± 2.20	5.50 ± 0.70	1.65 ± 0.07	0.42 ± 0.01	152.27
2	79.60 ± 1.03	3.80 ± 0.20	1.64 ± 0.05	0.60 ± 0.05	97.26
3	56.56 ± 0.62	8.96 ± 0.44	5.13 ± 0.09	0.50 ± 0.09	103.67
4	90.07 ± 2.14	4.37 ± 0.32	ND[b]	1.93 ± 0.06	105.24
5	50.85 ± 1.21	2.65 ± 0.70	ND[b]	1.58 ± 0.05	57.6
6	43.80 ± 0.42	2.70 ± 0.14	5.87 ± 0.17	ND[b]	66.32
7	64.55 ± 0.63	1.90 ± 0.14	ND[b]	0.65 ± 0.07	66.45
8	44.90 ± 0.14	7.05 ± 0.70	7.70 ± 0.11	0.34 ± 0.01	74.42
9	31.85 ± 0.49	2.70 ± 0.14	2.01 ± 0.14	2.21 ± 0.09	41.81
10	25.70 ± 0.42	2.20 ± 0.08	3.04 ± 0.08	1.59 ± 0.22	41.54
11	45.85 ± 0.35	3.65 ± 0.21	2.26 ± 0.02	0.47 ± 0.10	55.81
12	1.90 ± 0.14	ND[b]	ND[b]	ND[b]	4.50
13	22.75 ± 0.35	1.15 ± 0.07	12.45 ± 0.07	ND[b]	43.30
14	5.30 ± 0.14	1.60 ± 0.02	3.65 ± 0.06	1.00 ± 0.00	18.25
15	13.95 ± 0.07	7.20 ± 0.14	2.38 ± 0.09	0.29 ± 0.03	31.03
16	52.75 ± 0.35	5.15 ± 0.10	3.71 ± 0.07	ND[b]	74.96
17	10.83 ± 0.24	6.00 ± 0.141	1.89 ± 0.07	0.40 ± 0.06	22.81
18	79.8 ± 0.28	3.60 ± 0.14	3.84 ± 0.21	0.31 ± 0.06	99.09
19	24.90 ± 0.14	0.87 ± 0.04	2.35 ± 0.07	0.73 ± 0.08	33.53
20	54.75 ± 0.91	2.19 ± 0.15	ND[b]	0.99 ± 0.01	63.09
Average	46.7	3.6	2.9	0.6	53.8

Wine codes are as in Table1.
[a] Values represent means of triplicate determinations ($n = 3$) ± SD. Concentration is expressed as mg l^{-1}.
[b] ND: Not detected.

Table 4
Analytical hydroxycinnamate composition of the wines tested[a]

Wine codes	Caffeic acid	*p*-Coumaric acid	Ferulic acid	Total
1	9.92 ± 0.08	0.30 ± 0.01	0.19 ± 0.01	0.91
2	12.22 ± 0.31	0.78 ± 0.01	0.15 ± 0.01	1.53
3	33.02 ± 0.30	0.39 ± 0.01	3.95 ± 0.01	5.73
4	10.8 ± 0.28	1.02 ± 0.02	0.55 ± 0.07	3.50
5	4.10 ± 0.14	0.47 ± 0.03	0.33 ± 0.03	2.38
6	13.95 ± 0.21	1.73 ± 0.06	0.49 ± 0.01	2.22
7	ND[b]	ND[b]	ND[b]	0.65
8	14.77 ± 0.32	0.48 ± 0.03	0.83 ± 0.05	1.65
9	5.25 ± 0.21	1.55 ± 0.07	0.32 ± 0.14	4.08
10	10.60 ± 0.70	4.31 ± 0.05	0.62 ± 0.03	6.62
11	4.05 ± 0.21	2.76 ± 0.06	1.10 ± 0.01	4.33
12	2.60 ± 0.14	0.88 ± 0.21	ND[b]	0.88
13	6.95 ± 0.07	0.29 ± 0.01	0.51 ± 0.01	0.80
14	7.70 ± 0.28	1.59 ± 0.01	1.51 ± 0.02	4.10
15	7.50 ± 0.14	0.56 ± 0.02	0.78 ± 0.01	1.63
16	13.35 ± 0.63	1.04 ± 0.08	0.33 ± 0.03	1.37
17	4.54 ± 0.08	0.27 ± 0.07	0.21 ± 0.00	0.88
18	11.85 ± 0.21	0.67 ± 0.04	ND[b]	0.98
19	5.41 ± 0.12	1.11 ± 0.01	ND[b]	1.84
20	6.15 ± 0.07	2.25 ± 0.07	0.85 ± 0.07	4.09
Average	9.2	1.1	0.6	10.9

Wine codes are as in Table1.
[a] Values represent means of triplicate determinations ($n = 3$) ± SD. Concentration is expressed as mg l^{-1}.
[b] ND: Not detected.

reported for other countries by Sato et al. (1996). Regarding varieties 1–8, they all contained appreciable amounts of phenolic compounds with Thrapsa being the richest variety in total phenols, followed by Karvouniaris and Bakouri. Xinomavro was the second richest variety in total phenols, followed by Mandilaria, which is in agreement with the results obtained by Kallithraka et al. (2001). Both the above varieties are used for producing Greek wines having appel-

Table 5
Analytical flavanol composition of the wines tested[a]

Wine codes	Catechin	Epicatechin	Procyanidin				Total
			B1	B2	C1	A2	
1	112.83 ± 0.55	39.36 ± 0.52	29.08 ± 0.19	33.88 ± 0.10	7.95 ± 0.52	12.47 ± 0.01	235.59
2	200.5 ± 3.10	85.75 ± 0.77	22.96 ± 0.04	25.16 ± 0.31	6.63 ± 0.00	14.03 ± 0.04	355.03
3	120.35 ± 0.91	49.9 ± 0.14	7.47+/−0.53	ND[b]	ND[b]	21.23 ± 0.24	198.95
4	78.17 ± 0.53	73.12 ± 0.87	15.17 ± 0.46	33.34 ± 0.09	18.43 ± 0.26	ND[b]	218.24
5	107.35 ± 0.97	47.25 ± 0.35	9.39 ± 0.49	ND[b]	5.34 ± 0.44	ND[b]	169.33
6	53.32 ± 0.45	150.77 ± 1.08	34.19 ± 0.68	4.25 ± 0.04	19.54 ± 0.74	6.95 ± 0.05	269.03
7	144.65 ± 0.49	31.7 ± 0.56	ND[b]	ND[b]	14.35 ± 0.27	4.45 ± 0.39	209.25
8	11.65 ± 0.49	46.8 ± 0.28	38.07 ± 0.39	18.86 ± 0.68	51.17 ± 0.74	15.77 ± 0.19	195.15
9	37.35 ± 0.63	28.35 ± 0.35	4.17 ± 0.15	ND[b]	ND[b]	15.89 ± 0.99	38.85
10	7.17 ± 0.05	24.77 ± 0.32	9.94 ± 0.49	12.77 ± 0.65	46.10 ± 2.23	22.85 ± 1.67	67.50
11	32.83 ± 0.23	54.23 ± 1.08	12.96 ± 0.29	16.84 ± 0.68	23.69 ± 0.40	25.21 ± 0.29	182.32
12	ND[b]	32.90 ± 0.14	ND[b]	ND[b]	ND[b]	8.91 ± 0.87	85.77
13	14.40 ± 0.14	6.15 ± 0.06	8.14 ± 0.12	5.60 ± 0.02	ND[b]	4.55 ± 0.05	102.37
14	6.65 ± 0.21	40.20 ± 0.28	4.64 ± 0.41	3.61 ± 0.17	ND[b]	12.40 ± 0.11	75.14
15	13.35 ± 0.77	31.16 ± 1.18	3.91 ± 0.25	5.39 ± 0.32	15.19 ± 0.44	6.14 ± 0.23	41.81
16	21.71 ± 0.83	36.85 ± 0.64	6.42 ± 0.56	5.34 ± 0.29	27.98 ± 0.44	4.06 ± 0.22	59.10
17	10.65 ± 0.48	9.80 ± 0.14	7.81 ± 0.34	12.60 ± 0.41	11.37 ± 0.45	6.86 ± 0.43	123.61
18	53.2 ± 0.56	108.4 ± 1.84	16.90 ± 0.10	6.95 ± 0.05	17.16 ± 0.75	6.63 ± 0.02	129.15
19	36.62 ± 0.53	42.65 ± 0.35	6.78 ± 0.44	21.28 ± 0.56	17.62 ± 0.19	4.19 ± 0.75	165.76
20	41.90 ± 0.14	29.35 ± 1.06	9.48 ± 0.45	9.41 ± 0.70	31.84 ± 0.32	26.23 ± 0.98	148.22
Average	55.2	48.5	12.4	10.8	15.7	10.9	153.4

Wine codes are as in Table1.
[a] Values represent means of triplicate determinations ($n = 3$) ± SD. Concentration is expressed as mg l^{-1}.
[b] ND: Not detected.

Table 6
Analytical flavonol composition of the wines tested[a]

Wine codes	Myricetin	Kaempherol	Quercetin	Isokaempherol	Rutin	Total
1	1.61 ± 0.01	0.20 ± 0.01	2.22 ± 0.01	0.52 ± 0.00	15.01 ± 0.01	19.06
2	2.13 ± 0.04	2.47 ± 0.04	5.30 ± 0.07	1.73 ± 0.01	11.71 ± 0.24	21.61
3	2.44 ± 0.05	1.35 ± 0.07	6.49 ± 0.15	1.75 ± 0.10	15.18 ± 0.47	25.46
4	3.16 ± 0.10	7.20 ± 0.10	10.38 ± 0.20	1.76 ± 0.09	33.68 ± 1.25	54.43
5	1.31 ± 0.01	1.83 ± 0.07	4.41 ± 0.23	1.01 ± 0.05	3.85 ± 0.08	11.40
6	3.18 ± 0.09	1.88 ± 0.02	3.42 ± 0.11	1.63 ± 0.04	6.74 ± 0.44	15.23
7	ND[b]	ND[b]	1.67 ± 0.03	ND[b]	5.45 ± 0.27	36.05
8	31.90 ± 0.80	3.60 ± 0.14	10.55 ± 0.35	1.80 ± 0.06	20.08 ± 0.47	7.12
9	1.80 ± 0.14	1.00 ± 0.24	3.20 ± 0.14	1.03 ± 0.06	4.85 ± 0.20	47.19
10	21.35 ± 0.78	7.32 ± 0.30	16.07 ± 0.04	4.14 ± 0.06	10.60 ± 0.52	18.92
11	5.05 ± 0.35	1.00 ± 0.14	4.55 ± 0.07	ND[b]	7.02 ± 0.93	66.13
12	ND[b]	1.30 ± 0.02	2.33 ± 0.09	ND[b]	ND[b]	10.85
13	18.25 ± 0.35	8.06 ± 0.40	15.20 ± 0.24	4.59 ± 0.03	5.68 ± 0.05	26.95
14	7.45 ± 0.70	7.45 ± 0.21	6.65 ± 0.21	2.72 ± 0.05	4.37 ± 0.08	7.98
15	7.79 ± 0.60	3.67 ± 0.02	7.70 ± 0.12	1.01 ± 0.03	3.84 ± 0.45	3.64
16	2.10 ± 0.14	5.15 ± 0.21	9.85 ± 0.21	2.00 ± 0.14	9.85 ± 0.29	5.70
17	ND[b]	ND[b]	2.55 ± 0.07	ND[b]	3.15 ± 0.54	55.34
18	3.43 ± 0.09	6.00 ± 0.14	9.35 ± 0.21	2.56 ± 0.06	17.27 ± 0.10	21.51
19	2.23 ± 0.19	6.88 ± 0.12	4.90 ± 0.14	ND[b]	7.49 ± 0.51	17.62
20	1.20 ± 0.14	0.95 ± 0.40	3.27 ± 0.18	ND[b]	5.64 ± 0.47	11.06
Average	5.4	3.0	6.2	1.3	9.5	25.4

Wine codes are as in Table1.
[a] Values represent means of triplicate determinations ($n = 3$) ± SD. Concentration is expressed as mg l^{-1}.
[b] ND: Not detected.

lation of origin names. Furthermore, Agiorgitiko (also used for wines with appellation of origin names) contained similar total phenolic content as the rare native varieties. Wines made from the international varieties Syrah and Cabernet Sauvignon (Nos. 13 and 14, respectively) contained intermediate amounts of total phenols. Finally,

Table 7
Analytical anthocyanin composition of the wines tested[a]

Wine codes	Delphinidin	Cyanidin	Petunidin	Peonidin	Malvidin	Malv-acetate	Malv-coumarate
1	ND[b]	ND[b]	5.40 ± 0.47	2.68 ± 0.40	126.22 ± 0.34	31.88 ± 0.26	28.05 ± 0.86
2	7.57 ± 0.20	ND[b]	15.02 ± 0.33	19.0 ± 0.53	271.94 ± 0.96	13.54 ± 0.62	25.14 ± 0.31
3	8.01 ± 0.15	9.57 ± 0.58	17.90 ± 0.34	58.14 ± 0.32	198.92 ± 0.74	1.93 ± 0.04	17.50 ± 0.69
4	5.43 ± 0.32	ND[b]	12.71 ± 0.58	12.55 ± 0.61	272.94 ± 0.38	7.63 ± 0.09	36.19 ± 0.58
5	ND[b]	ND[b]	1.16 ± 0.07	ND[b]	7.87 ± 0.02	2.78 ± 0.11	6.78 ± 0.16
6	5.76 ± 0.09	ND[b]	15.01 ± 0.12	7.71 ± 0.24	339.18 ± 0.27	58.79 ± 0.27	36.66 ± 0.21
7	ND[b]	ND[b]	ND[b]	1.59 ± 0.04	17.70 ± 0.40	ND[b]	1.71 ± 0.05
8	51.46 ± 0.08	3.62 ± 0.04	72.71 ± 0.02	14.07 ± 0.07	303.26 ± 0.38	8.06 ± 0.09	43.29 ± 0.10
9	ND[b]	ND[b]	ND[b]	2.23 ± 0.09	45.10 ± 0.77	ND[b]	10.58 ± 0.62
10	74.31 ± 0.46	22.01 ± 0.20	122.34 ± 0.48	73.20 ± 0.23	534.24 ± 0.94	82.26 ± 0.35	103.47 ± 0.68
11	5.27 ± 0.07	ND[b]	13.26 ± 0.28	3.24 ± 0.06	143.34 ± 0.89	31.34 ± 0.07	16.02 ± 0.08
12	2.46 ± 0.05	ND[b]	10.61 ± 0.24	4.43 ± 0.02	249.68 ± 0.10	22.82 ± 0.10	34.88 ± 0.09
13	2.87 ± 0.15	ND[b]	15.35 ± 0.34	8.58 ± 0.29	252.10 ± 0.16	115.77 ± 0.56	63.25 ± 0.37
14	19.78 ± 0.66	ND[b]	31.16 ± 0.69	10.84 ± 0.68	415.98 ± 0.38	186.09 ± 0.86	35.08 ± 0.26
15	ND[b]	ND[b]	ND[b]	ND[b]	17.10 ± 0.03	ND[b]	3.05 ± 0.04
16	2.17 ± 0.02	ND[b]	4.359 ± 0.09	5.39 ± 0.08	70.02 ± 1.23	11.39 ± 0.38	11.54 ± 0.21
17	ND[b]	ND[b]	ND[b]	ND[b]	22.78 ± 0.03	ND[b]	ND[b]
18	2.89 ± 0.10	ND[b]	8.40 ± 0.02	5.90 ± 0.10	311.26 ± 0.39	27.75 ± 0.24	46.50 ± 0.35
19	1.94 ± 0.11	ND[b]	4.51 ± 0.07	4.36 ± 0.04	116.23 ± 0.70	12.46 ± 0.10	21.32 ± 0.54
20	9.34 ± 0.14	ND[b]	20.59 ± 0.71	21.35 ± 0.14	361.25 ± 0.75	24.37 ± 0.44	41.51 ± 0.35
Average	9.9	1.8	18.5	12.8	203.8	31.9	29.1

Wine codes are as in Table1.
[a] Values represent means of triplicate determinations ($n = 3$)±SD. Concentration is expressed as mg l^{-1}.
[b] ND: Not detected.

wines made from Mavro Mesenikola, Krasato, Refosko and Barbera had the lowest total phenolic values.

Regarding total anthocyanin content (TA) (Table 2), it varied from 19 (No. 5) to 1012, (No. 10) the average being 308 mg l^{-1} in agreement with the results obtained by Harvalia and Bena-Tzourou (1982). Vertzami was the richest variety in TA, followed by Cabernet Sauvignon and Augoustiati. Mandilaria and Agiorgitiko were also rich in TA, in agreement with the results of Kallithraka et al. (2001) and Makris et al. (2002). In contrast, Kotselina, Voidomatis, Krasato and Mavro Mesesnikola were the varieties with the lowest TA contents. The TA content of the rest of the rare native varieties, with the exception of Karvouniaris, was close to the average.

3.2. Determination of individual polyphenolic composition

Four phenolic acids including gallic acid, protocatechuic acid, syringic acid and vanillic acid could be separated and quantified by the HPLC method employed (Table 3). Gallic acid (mean concentration 46.7 mg l^{-1}) was by far the predominant acid, as it represented 86.8% of all phenolic acids. Protocatechuic acid was the second most abundant acid found (mean 3.6 mg l^{-1}) followed by syringic acid (2.9 mg l^{-1}) but vanillic acid was a minor constituent, its mean concentration being 0.6 mg l^{-1}. These results are in agreement with Arnous et al. (2002b) regarding Greek wines as well as with Minussi et al. (2003) regarding international wines. As it can be seen from Table 3, Karvouniaris (No. 1, Table 1) was the richest variety in total phenolic acid composition followed by Bakouri (No. 4), Nerostafilo (No. 3), Agiorgitiko (No. 18) and Thrapsa (No. 2). The rest of the rare native varieties were also rich in phenolic acids. The varieties with the lowest amounts of phenolic acids were Refosko (No. 12), Cabernet Sauvignon (No. 14), Mavro Mesesnikola (No. 17) and Krasato (No. 15).

With respect to hydroxycinnamates, three acids were quantified: caffeic, *p*-coumaric and ferulic acids (Table 4). Mean concentrations of caffeic (9.2 mg l^{-1}) and coumaric (1.1 mg l^{-1}) acids were in agreement with the results of Arnous et al. (2002a) and Minussi et al. (2003). Vertzami (No. 10 in Table 1), was the richest variety in hydroxycinnamates, followed by Nerostafilo (No. 3), Barbera (No. 11), Cabernet Sauvignon (No. 14) and Mandilaria (No. 20). The varieties with the lowest amounts were Voidomatis (No. 7), Syrah (No. 13), Refosko and Mavro Mesenikola (Nos. 12 and 17, respectively).

Catechin and epicatechin were two of the major flavonoid compounds detected in wines (Table 5), having mean concentrations of 55.2 and 48.5 mg l^{-1}, respectively. These results are in agreement with previous findings (Arnous et al., 2002a; Kallithraka et al., 2001). Regarding procyanidins, C1 was the major one (mean concentration 15.7 mg l^{-1}) followed by B1 (mean 12.4 mg l^{-1}), A2 (mean 10.9 mg l^{-1}) and B2 (mean 10.8 mg l^{-1}). At this point, it should be mentioned that this is the first time that the concentration of procyanidins B2 and A2 has been determined and reported in Greek wines. Thrapsa (No. 2 in Table 1) was the richest variety in total flavanols, followed by Karvouniaris, Limniona, Bakouri and Voidomatis (Nos. 1, 6, 4 and 7, respectively). With the exception of Vertzami, all

rare native varieties were particularly rich in flavanols. In contrast, Moshato Amvourgou (No. 9) was the poorest variety in total flavanols followed by Krasato, Stavroto and Vertzami (Nos. 15, 16 and 10, respectively).

Five flavonols were detected: myricetin (mean concentration 5.4 mg l^{-1}), kaempherol (mean 3 mg l^{-1}), quercetin (mean 6.2 mg l^{-1}), isokaempherol (mean 1.3 mg l^{-1}) and rutin (mean 9.5 mg l^{-1}) (Table 6). These mean values fell within the range reported for other countries (McDonald et al., 1998; Simonetti, Pietta, & Testolin, 1997) as well as for Greece (Kallithraka et al., 2001). Barbera (No. 11 in Table 1) was the variety with the highest concentration of total flavonols, followed by Mavro Mesenikola (No. 17), Bakouri (No. 4) and Moshato Amvourgou (No. 9). The lowest concentrations were found for Krasato, Stavroto and Augoustiatis (Nos. 15, 16 and 8, respectively).

With regard to anthocyanins, it can be seen that, apart from the five standard anthocyanins, another two were consistently detected. These compounds were quantified as malvidin 3-*O*-glucoside. According to previously published data (Arnous et al., 2002a) these two compounds should be malvidin-3-*O*-glucose acetate and *p*-coumarate. This assumption is supported by the fact that the second one also absorbed at 320 nm, indicating the existence of an hydroxycinnamate moiety (Roggero, Coen, & Ragonnet, 1986), but this was not detected for the first one. The quantitative determination showed that malvidin-3-*O*-glucoside was the predominant anthocyanin (mean concentration 203.8 mg l^{-1}) followed by malvidin-3-*O*-glucoside acetate. In contrast, cyanidin-3-*O*-glucoside had the lowest mean content (1.8 mg l^{-1}) followed by delphinidin (9.96 mg l^{-1}). These results are in agreement with previous findings (Arnous et al., 2002a; Kallithraka et al., 2001).

3.3. General discussion

The examination of the analytical polyphenolic composition has in some instances provided evidence for the potential of certain cultivars for polyphenol biosynthesis. The results obtained confirm a variation in phenolic content among wine samples tested. The range of the data obtained is in agreement with the available international literature (Frankel, Waterhouse, & Teissedre, 1995; Goldberg & Soleas, 1999; Kanner, Frankel, Granit, German, & Kinsella, 1994; Sato et al., 1996; Simonetti et al., 1997; Soleas, Dam, Carey, & Goldberg, 1997). It is irrefutable that the amounts as well as the various species of phenolics that occur in wines depend on a wide range of factors, including cultural practices, local climate conditions, vinification techniques and storage and aging (Frankel et al., 1995). These factors make comparisons between different wines difficult. The results presented in this paper are indicative of the polyphenolic richness of certain cultivars since enviromental factors (in 12 of the cases) and enological techniques (in all cases) were kept constant. At this point it should be mentioned that the experimental wines tested were produced under similar conditions, in order to make possible a comparison between them, and not under the optimal enological conditions required for every single variety. The potential of each variety to produce quality wines must be explored separately by selecting the appropriate eonological practices (such as skin contact time, temperature, etc.) for production at industrial scale.

The critical assessment of the data from the wines analysed clearly indicates some cultivars to be distinctive for their exceptional polyphenolic potential. Thrapsa, Karvouniaris, Vertzami, Bakouri and Augoustiatis were particularly rich in both flavonoid and non-flavonoid phenolics. Their phenolic content was comparable, and in some cases richer, than the content of the most known varieties used for producing quality wines with appellation of origin names (Agiorgitiko, Xinomavro, Mandilaria). The above mentioned rare native varieties were also richer in polyphenols than the international ones, Syrah and Cabernet Sauvignon. In addition, Vertzami was the variety with the exceptional high total anthocyanin content. Given its also high phenolic content, this variety could be possibly used in mixtures with varieties poor in TA (e.g., Voidomatis, Kotselina, Krasato, Mavro Mesenikola) to produce balanced, in both flavor and color, wines. Augoustiatis could also be used in mixtures to enrich the poor in TA and TP varieties.

The analytical composition data that have been available to date also permit the distinction of trends related to specific phenolic metabolites, and therefore allow recognition of variety-dependent patterns. Regarding the three most famous Greek varieties (Agiorgitioko, Xinomavro, Mandilaria), the same trend was observed as in previous studies (Arnous et al., 2001; Arnous et al., 2002b; Kallithraka et al., 2001; Sakkiadi et al., 2001). Wines made from Agiorgitiko and Mandilaria were rich in anthocyanins, but wines made from Xinomavro were rich in flavanols

Table 8
Percent of variance explained by the first three principal components[a]

Component	Eigenvalue	% of Variance	Cumulative (%)
1	13.22	66.11	66.11
2	2.70	13.50	79.62
3	1.87	9.35	88.98

[a] Extraction method: principal component analysis of phenolic compounds of red wines.

Table 9
Percent of variance explained by the first three principal components[a]

Component	Eigenvalue	% of Variance	Cumulative (%)
1	19.00	95.01	95.01
2	0.57	2.86	97.87
3	0.31	1.55	99.43

[a] Extraction method: principal component analysis of anthocyanins of red wines.

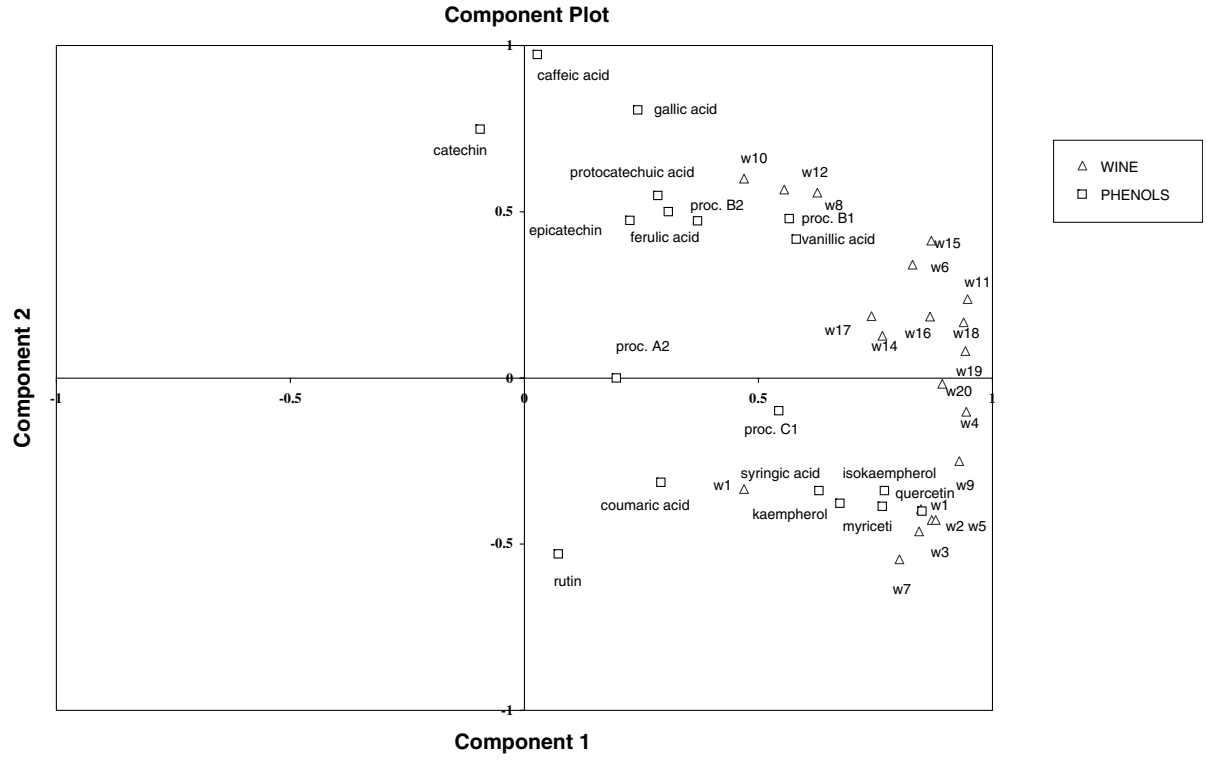

Fig. 1. Principal component analysis of phenolic compounds of red wines (PC1 vs. PC2). For wine codes see Table 1.

in spite of their low anthocyanin content. Furthermore, Karvouniaris, Thrapsa and Augoustiatis were high in phenolic acids and flavanols but low in flavonols and hydroxycinnamic acids. Nerostafilo and Bakouri were also rich in phenolic acids and flavanols but the former was poor in flavanols and the latter poor in hydroxycinnamates.

PCA was applied to the data of the wines in order to obtain any differentiation based on their phenolic and/or

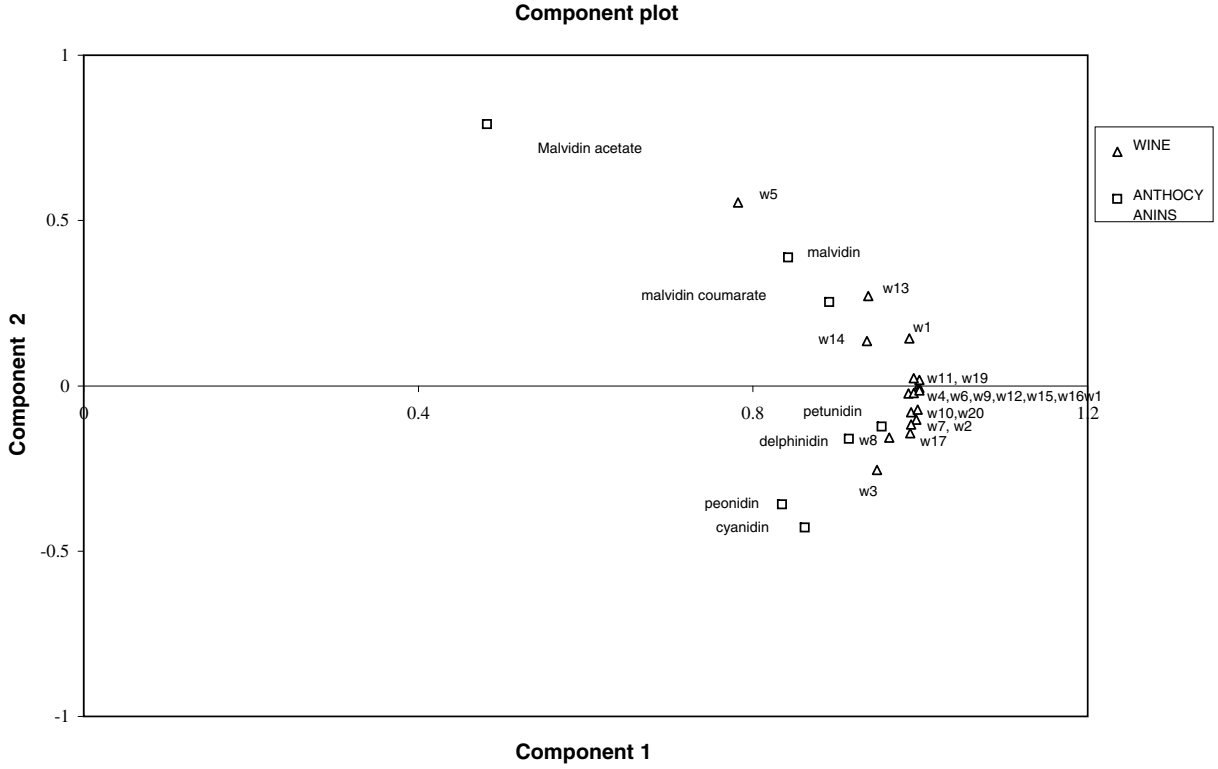

Fig. 2. Principal component analysis of anthocyanins of red wines (PC1 vs. PC2). For wine codes see Table 1.

anthocyanin content. Tables 8 and 9 depict the cumulative percentage of the total variance explained by the first three principal components. The first two factors retained 79.6% of the variance in Table 8 and 97.85% in Table 9.

Based on wine phenolic content, some grouping could be observed in the space formed by the two first components (Fig. 1). Group A (wines 8, 10 and 12) could be best described by parameters such as procyanidin B1, procyanidin B2 and vanillic acid. Group B, which consists mainly from the rare native Greek varieties (wines 1, 2, 3, 4, 5, 7 and 9) could be best described by the flavonols quercetin, myricetin, isokaempherol and kaempherol. Finally, group C, which mainly consists of the international varieties as well as the varieties used to produce appellation of origin wines (wines 6, 11, 14, 15, 16, 17, 18, 19 and 20) could not be characterized by any of the phenolic parameters used in this study.

In Fig. 2, most of the wines fell into the same area, since it appeared as if there were no major differences among them. The main parameters expressing this grouping were petunidin and delphinidin. Exceptions were wines 1, 5, 13 and 14, which could be best described by malvidin and malvidin coumarate, and wine 3.

In conclusion, some of the unexploited rare native varieties were found to contain appreciable amounts of non-colored phenols as well as anthocyanins so that they would be worthy of further study and use for the production of quality wines. For example, Karvouniaris, Thrapsa, Nerostafilo, Bakouri and Vertzami might have the potential to be used by the Greek wine industry, either alone or in mixtures to enrich the phenolic content and improve the color of other varieties. Furthermore, the data revealed valuable information regarding the variety-related patterns for major phenolics in autochthonous cultivars. Karvouniaris, Thrapsa and Augoustiatis were found high in phenolic acids and flavanols but low in flavonols and hydroxycinnamic acids. Nerostafilo and Bakouri were also rich in phenolic acids and flavanols but the former was poor in flavanols and the latter poor in hydroxycinnamates. PCA of the results showed that the wines could be grouped in three major classes depending on their phenolic content.

References

Arnous, A., Makris, D. P., & Kefalas, P. (2001). Effect of principal polyphenolic components in relation to antioxidant characteristics of aged red wines. *Journal of Agricultural and Food Chemistry, 49*, 5736–5742.

Arnous, A., Makris, D. P., & Kefalas, P. (2002a). Anthocyanin composition and colour characteristics of selected aged wines produced in Greece. *Journal of Wine Research, 13*(1), 23–34.

Arnous, A., Makris, D. P., & Kefalas, P. (2002b). Correlation of pigment and flavanol content with antioxidant properties in selected aged regional wines from Greece. *Journal of Food Composition and Analysis, 15*(6), 655–665.

Cheynier, V., & Ricardo Da Silva, J. M. (1991). Oxidation of grape procyanidins in model solutions containing *trans*-caffeoyl-tartaric acid and polyphenol oxidase. *Journal of Agricultural and Food Chemistry, 39*, 1047–1049.

Dourtoglou, V. G., Yannovits, N. G., Tychopoulos, V. G., & Mamvakias, M. M. (1994). Effect of storage under CO_2 atmosphere on the volatile, amino acid, and pigment constituents in red grape (*Vitis vinifera* L. Var. Agiorgitiko). *Journal of Agricultural and Food Chemistry, 42*, 338–344.

Frankel, E. N., Kanner, J. B., German, E., & Kinsella, J. E. (1993). Inhibition of human low-density lipoprotein by phenolic substances in red wine. *Lancet, 341*, 454–457.

Frankel, E. N., Waterhouse, A. L., & Teissedre, P. L. (1995). Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. *Journal of Agricultural and Food Chemistry, 43*, 890–894.

Goldberg, D. M., & Soleas, G. (1999). Analysis of antioxidant wine polyphenols by high performance liquid chromatography. *Analytical Chemistry, 29*, 122–137.

Harvalia, A., & Bena-Tzourou, I. (1982). Etude de quelques facteurs influencant la couleur des vins rouges Helleniques. *Connaissance de la vigne et du vin, 61*, 1–13.

Haslam, E. (1980). In vino veritas: oligomeric procyanidins and the aging of red wines. *Phytochemistry, 19*, 1577–1582.

Kallithraka, S., Arvanitoyannis, I., El-Zajouli, A., & Kefalas, P. (2001). The application of an improved method for *trans*-resveratrol to determine the origin of Greek red wines. *Food Chemistry, 75*, 355–363.

Kallithraka, S., Arvanitoyannis, I., Kefalas, P., El-Zajouli, A., Soufleros, E., & Psarra, E. (2001). Instrumental and sensory analysis of Greek wines; implementation of principal component analysis (PCA) for classification according to geographical origin. *Food Chemistry, 73*, 501–514.

Kallithraka, S., Mohdaly, A. A., Makris, D. P., & Kefalas, P. (2005). Determination of major anthocyanin pigments in Hellenic native grape varieties (*Vitis vinifera* sp.): association with antiradical efficiency. *Journal of Food Composition and Analysis, 18*, 375–386.

Kanner, J., Frankel, E. N., Granit, R., German, B., & Kinsella, J. E. (1994). Natural antioxidants in grapes and wines. *Journal of Agricultural and Food Chemistry, 42*, 64–69.

Kato, R., Nakadate, I., Yamamoto, S., & Sigimura, T. (1983). Inhibition of 12-*O*-tetradecanoylphorbol-13-acetate-induced tumor promotion and ornithine decarboxylase activity by quercetin: possible involvement of lipoxygenase inhibition. *Carcinogenesis, 4*, 1631–1637.

Knekt, P., Jarvinen, R., Reunanen, A., & Maatela, J. (1996). Flavonoid intake and coronary mortality in Finland: a cohort study. *British Medical Journal, 312*, 478–481.

Lanaridis, P., & Bena-Tzourou, E. (1997). Étude des variations des anthocyanes pendant la maturation des raisins de cinq cépage rouges, cultivé en Grèce. *Journal International des Sciences de la Vigne et du Vin, 31*(4), 205–212.

Makris, D. P., Psarra, E., Kallithraka, S., & kefalas, P. (2002). The effect of polyphenolic composition as related to antioxidant capacity in white wines. *Food Research International, 36*, 805–814.

McDonald, M. S., Hughes, M., Burns, M., Lean, J., Mathews, D., & Crozier, A. (1998). Survey of the free and conjucated myricetin and quercetin content of red wines of different geographical origins. *Journal of Agricultural and Food Chemistry, 46*, 368–375.

Mehanso, H., Butler, L. G., & Carlson, D. M. (1987). Dietary tannins and salivary proline-rich proteins: interactions, induction and defense mechanisms. *Annual Review of Nutrition, 7*, 423–440.

Minussi, R. C., Rossi, M., Bologna, L., Cordi, L., Rotilio, D., Pastore, G. M., et al. (2003). Phenolic compounds and total antioxidant potential of commercial wines. *Food Chemistry, 82*, 409–416.

Ozmianski, J., & Sapis, J. C. (1989). Fractionation and identification of some low molecular weight grape seed phenolics. *Journal of Agricultural and Food Chemistry, 37*, 1293–1297.

Ricardo Da Silva, J. M., Cheynier, V., Souquet, J. M., Moutonet, M., Cabanis, J. C., & Bourzeix, M. (1991). Interaction of grape seed proteins in relation to wine fining. *Journal of Science and Food Agriculture, 57*, 111–125.

Roggero, J. P., Coen, S., & Ragonnet, B. (1986). High performance liquid chromatography survey on changes in pigment content in ripening

grapes of Syrah. An approach to anthocyanin metabolism. *American Journal of Enology and Viticulture, 37*, 77–83.

Robichaud, J. L., & Noble, A. C. (1990). Astringency and bitterness of selected phenolics in wine. *Journal of Science and Food Agriculture, 53*, 343–353.

Sakkiadi, A. V., Haroutounian, S. A., & Stavrakakis, M. N. (2001). Direct HPLC assay of five biologically interesting phenolic antioxidants in varietal Greek red wines. *Lebensmittel-Wissenschaft und-Technologie, 34*, 410–413.

Sato, M., Ramarathnam, N., Suzuki, Y., Okhudo, T., Takeuchi, M., & Ochi, H. (1996). Varietal differences in the phenolic content and superoxide radical scavenging potential of wines from different sources. *Journal of Agricultural and Food Chemistry, 44*, 37–41.

Simonetti, P., Pietta, P., & Testolin, G. (1997). Polyphenol content and total antioxidant potential of selected Italian wines. *Journal of Agricultural and Food Chemistry, 45*, 1152–1255.

Soleas, G., Dam, J., Carey, M., & Goldberg, D. (1997). Toward the fingerprinting of wines: cultivar-related patterns of polyphenolic constituents in Ontario wines. *Journal of Agricultural and Food Chemistry, 45*, 3871–3880.

SPSS Base 9.0 User's Guide (1999). *SPSS for windows 98*, Chicago, USA.

Verma, A., Johnson, J. A., Gould, M. N., & Tanner, M. A. (1988). Inhibition of 7,12-dimethylbenzanthracene and *N*-nitrosomethylurea induced rat mammary cancer by dietary flavonol quercetin. *Cancer Research, 48*, 5754–5758.

Waterman, P. G., & Mole, S. (1994). *Analysis of phenolic plant metabolites*. Oxford: Blackwell Scientific Publ. (pp. 83–91).