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Study of polyphenol content in the seeds of red grape (*Vitis vinifera* L.) varieties cultivated in Turkey and their antiradical activity

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Abstract

The polyphenolic content and antioxidant capacity in the seeds of 11 red grape varieties (five international and six native) widely cultivated in Turkey were investigated. Total phenolic, total flavanol and total polymeric procyanidin content ranged from 79.2 to 154.6, 89.2 to 179.4, and 27.0 to 43.3 mg/g seed, respectively. While (+)-catechin (4.71–23.8 mg/g seed) was found as main flavanol, galloylated catechin monomer and dimeric procyanidin amounts varied between 2.89–17.2 and 0.97–2.97 mg/g seed, respectively. All seed extracts showed remarkable DPPH radical scavenging activity (EC_{50}) and oxygen radical scavenging capacity (ORAC) ranging from 2.71 to 4.62 μg/mL and 1425.9 to 3009.2 μmol Trolox equivalent/g seed, respectively. With high amount of total phenolic content and antioxidant activity, seeds of Okuzgozu, Papaz Karasi, Ada Karasi and Kalecik Karasi varieties could be evaluated as dietary supplement.
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Keywords: Grape seed; Total phenolics; Flavanol; Proanthocyanidin; DPPH; ORAC; HPLC

1. Introduction

In recent years, there has been a growing interest in the use of grape seed extracts as a dietary antioxidant supplements (Santos Buelgo & Scalbert, 2000). The antioxidant capacity, therefore, the health benefit of grape seed extracts are mainly due to their flavanols and proanthocyanidin (condensed tannin) contents. Proanthocyanidins constitute a complex mixture of monomers, oligomers and polymers which generally consists of (+)-catechin, (−)-epicatechin, (+)-gallocatechin, (−)-epigallocatechin and their 3-O-gallic acid esters (Prieur, Rigaud, Cheynier, & Moutounet, 1994). Proanthocyanidins are also important sensory components, providing especially red wine with bitterness and astringency (Robichaud & Noble, 1990), and responsible for antioxidant efficiency of wine products (Kanner, Frankel, Granit, German, & Kinsella, 1994; Renaud & De Lorgeril, 1992).

Turkey, today, is the fifth-largest producer of grapes and becoming one of the important wine producers in the world. In recent years, the market for wine has been expanding as more varieties and better quality wines become available, however, today almost 10% of grape has been used for wine making (Hodgen, 2005; Paul, 2006). Others have been using as table grapes, grape juice and dried grape production.

Although several studies (Bakkalbasi, Yemis, & Aslanova, 2005; Gurbuz et al., 2007; Orak, 2007) reported the major flavanol contents (catechin and epicatechin) and antioxidant activities (on DPPH free radical), the literature lacks information on detailed phenolics composition and ORAC antioxidant capacities of red grape seed varieties in Turkey. Therefore, the objective of this study was to determine phenolics composition and antiradical activities of major red grape seed varieties (native and international), which are widely used for wine making in Turkey.

2. Materials and methods

All red grape samples (native or international) were harvested at optimum maturity. Merlot, Cabernet Sauvignon, Cinsault, Alphonso Lavallee, Papaz Karasi, Muscat

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Hamburg, Ada Karasi, Senso were from Tekirdag Vineyard Research Institute, Okuzgozu and Bogazkere from commercial vineyard in Malatya (Arapkiri village) and Kalecik Karasi from Ankara. Seeds were manually separated, freeze dried, and kept below at −20 °C until used.

Freeze-dried seeds were ground by using a grinder for 10 s. Fatty material was removed by Soxhlet extraction using *n*-hexane for 6 h. Phenolic compounds were extracted from 2 g of defatted seeds with 50 mL of 0.5% acetic acid in acetone:water (70:30) at 50 °C in a shaker bath for 2 h. Extraction procedure was repeated in triplicate and combined extracts were evaporated. Remaining aqueous phase was freeze dried.

2.1. Determination of total phenolic, total flavanol and total proanthocyanidin content

Total phenolic (TP) content in the extracts was determined by the Folin-Ciocalteau colorimetric method (Prior et al., 1998), and expressed as gallic acid (mg GAE/g seed) equivalents. Total flavanol (TF) content was estimated using Vanillin-HCl method (Price, Scoyoc, & Butler, 1978). (+)-Catechin was used to establish standard curve (correlation coefficient, $r = 0.999$). Total flavanol was expressed as (+)-catechin (mg catechin/g seed) equivalent. Total polymeric proanthocyanidins with BuOH–HCl assay were determined according to the method of Cheynier, Labarbe, and Moutounet (2001). Proanthocyanidin dimer B1 (0.02–0.1 mg) is used to establish the standard curve (correlation coefficient, $r = 0.998$). Total proanthocyanidin content was expressed as Proanthocyanidin dimer B1 (mg Proanthocyanidin dimer B1/g seed) equivalent.

2.2. HPLC analysis of monomeric and dimeric flavanols

(+)-Catechin (CT), (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epigallocatechin gallate (EGCG), (−)-epicatechin gallate (ECG), proanthocyanidin B1 and B2 were determined by HPLC-DAD system (Agilent 1100 series, Waldbronn, Germany) described by Monagas, Gomez-Cardoves, Bartoloma, Laureano, and Da Silva (2003) with some modifications. For the separation, a 250 × 4.6 mm i.d., 5 μm, Kromsil C_{18} (Technocroma, Barcelona, Spain) operating at 30 °C was employed. The eluent was composed of (A) H_2O/CH_3COOH (98:2) and (B) $CH_3CN/H_2O/CH_3COOH$ (80:19.6:0.4), and the flow rate was 1 ml/min. The following linear gradient programme was used for the elution: from 0% to 10% B in 5 min, from 15% to 30% B in 20 min, from 30% to 50% B in 10 min, from 50% to 60% B in 5 min, and 60% to 90% B in 5 min followed by a return to the initial conditions in 5 min and re-equilibration of the column. Chromatogram was monitored at 280 nm. Identification was based on comparing retention times and on-line spectral data in comparison with original standards. Quantification was performed using the calibration curves of each standard compounds. Two determinations were made on each extracts obtained from two seed samples.

2.3. Determination of antiradical activity

The antiradical activity of grape seed extracts was measured using DPPH method (Sanchez-Moreno, Larrauri, & Saura-Calixto, 1998), and expressed as EC_{50} (μg/mL), the concentration necessary for 50% reduction of $DPPH^{\cdot}$.

Oxygen radical absorbance capacity of the extracts was determined by the ORAC-fluorescein assay with slight modification of the method presented (Prior et al., 2003). Trolox standards (12.5–400 μM), extract (0.01–0.015 mg/mL), fluorescein (10.0 nM), and 2,2-azobis (2-methylpropionamidine) dihydrochloride (AAPH) (240 nM) solutions were prepared prior to use in phosphate buffer (75 mM, pH 7.4). ORAC analyses were performed in a 96-well microplate fluorometer (FLUOSTAR Optima, BMG Lab, England). Fluorescence filters were used for an excitation wavelength of 485 nm and an emission wavelength of 520 nm. Fluorescence was measured every two minutes for 120 min. For each compound and each concentration measurements were made in four times. The final ORAC values were calculated using the net area under the decay curves and were expressed as μmol of Trolox Equivalents (TE) g/seed.

2.4. Statistical analysis

The data were subjected to ANOVA test (STATGRAPHICS Plus 3.1). Multiple comparison of the means was performed by least significant difference (LSD) test at $\alpha = 0.05$ level.

3. Results and discussion

3.1. Total phenolic, total flavanol, total proanthocyanidin and individual monomeric and dimeric flavanol content

Total extractable matter, total phenolic substance (TP), total flavanol (TF) and total proanthocyanidin content of grape seeds examined are illustrated in Table 1.

Extraction yield (g extract/100 g seed) ranged from 20.68 to 29.87. Total phenolic content measured by the Folin Ciocalteau was highest in Papaz Karasi (154.6 mg GAE/g seed), followed by Okuzgozu (139.4 mg GAE/g seed). All seeds from international varieties (Merlot, Cabernet Sauvignon, Cinsault and Alphonso Lavallee) contained moderate amount of TP (88.11–105.7 mg GAE/g seed).

Total monomeric and oligomeric flavanol (TF) content estimated by Vanillin-HCl assay showed almost similar trend as for the total phenolic content in grape seed varieties. TF was higher in Papaz Karasi (179.4 mg/g seed) and Okuzgozu (174.5 mg/g seed), and the lowest in Senso (89.2 mg/g seed) and it was similar in Alphonso Lavallee, Merlot and Cabernet Sauvignon (122.3–125.0 mg/g seed)

Table 1
Extractable matter, total phenolics and total flavanol and total polymeric procyanidin content of the grape seeds

Varieties	Extractable matter (g/100 g seed)	Total phenol (TP) (mg GAE/g seed)[A]	Total flavanol (TF) (mg CT/g seed)[B]	Total polymeric proanthocyanidin (PA) (mg B1/g seed)[C]
Merlot	21.7 ± 0.3^{ab}	105.7 ± 6.6^{c}	122.7 ± 5.8^{c}	33.1 ± 0.5^{c}
Cabernet	21.9 ± 0.4^{ab}	103.7 ± 5.5^{c}	125.0 ± 4.9^{c}	29.4 ± 1.1^{b}
Cinsault	20.7 ± 0.2^{a}	88.1 ± 5.5^{b}	97.1 ± 6.4^{ab}	27.2 ± 1.1^{a}
Papaz Karasi	29.2 ± 0.3^{d}	154.6 ± 9.1^{e}	179.4 ± 12.6^{f}	43.3 ± 1.2^{e}
Ada Karasi	25.2 ± 1.7^{c}	137.5 ± 9.6^{d}	163.4 ± 9.7^{e}	38.0 ± 3.1^{d}
Hamburg Muscat	24.2 ± 0.9^{c}	104.4 ± 6.9^{c}	105.7 ± 3.9^{b}	31.1 ± 1.8^{bc}
Alphonso Lavallee	23.3 ± 0.4^{bc}	105.3 ± 6.1^{c}	123.3 ± 8.1^{c}	31.3 ± 1.0^{bc}
Okuzgozu	29.9 ± 1.7^{d}	139.4 ± 13.9^{d}	174.5 ± 14.6^{f}	39.7 ± 4.7^{d}
Bogazkere	24.2 ± 1.1^{c}	94.2 ± 8.3^{b}	95.0 ± 7.8^{a}	32.1 ± 1.5^{c}
Senso	20.8 ± 1.9^{a}	79.2 ± 5.6^{a}	89.2 ± 5.6^{a}	27.0 ± 2.6^{a}
Kalecik Karasi	29.8 ± 0.1^{d}	136.2 ± 9.0^{d}	147.7 ± 10.8^{c}	38.0 ± 3.1^{d}

Values are means ± SD on the basis of dry seed. Different letters (a–f within the column show significant differences at $p < 0.05$).
[A] TP by Folin-Ciocaltaeu.
[B] TF by Vanillin-HCl.
[C] PA by BuOH-HCl.

($p > 0.05$). These findings were in consistency with the previous work (Orak, 2007) except Bogazkere variety. Surprisingly, Bogazkere, known as rich in polyphenol, had little TP (94.2 mg GAE/g seed) and TF (95.0 mg catechin/g seed).

Total polymeric proanthocyanidin (PA) content estimated with Bate-Smith (BuOH-HCl) reaction varied widely among the grape varieties, ranging from 27.0 to 43.3 mg/g seed. Native grape varieties (except Senso) had remarkable amount PA compared to that of international varieties (Table 1). Higher polymeric proanthocyanidin content was in Papaz Karasi, comprising 28% of the total phenolic content. Although lower total phenolic was found in Bogazkere, it contained a higher proportion of PA (34.07% of total phenolic).

Individual flavanol content (CT, EC, ECG, EGC, EGCG, proanthocyanidins B1 and B2) determined by HPLC-DAD are presented in Table 2. Large differences were found among the varieties in relation to the flavanol content. The main compound was catechin, with the exception of Merlot, Papaz Karasi and Senso in which (-)epicatechin was more abundant. Dimeric proanthocyanidins B1 and B2 were minor constituents in all varieties, and their average contents were 0.98 and 0.90 mg/g seed, respectively. Total galloylated catechin concentrations varied widely among the varieties, ranging from 2.66 to 17.2 mg/g seed.

As can be seen in Table 2, the amounts of flavanols present in native varieties were higher than those in international varieties. Varieties including Okuzgozu, Papaz Karasi and Kalecik Karasi were had significantly higher total flavanol content ($p < 0.05$), as opposed to Senso, Bogazkere and Ada Karasi, which were found to be particularly poor. By contrast the other studies (Bakkalbasi et al., 2005; Gurbuz et al., 2007), while catechin and epicatechin contents were lowest in Bogazkere, they were found to be higher in Okuzgozu, Kalecik Karasi and Papaz Karasi varieties. These differences may be due to the different solvent and procedure employed for the extraction of seeds. Total galloylated flavanol content were significantly higher in these varieties, being 21.14%, 33,61% and 30.07% of total flavonols determined by HPLC, for Kalecik Karasi, Papaz Karasi and Okuzgozu, respectively.

Varieties including Merlot, Cabernet Sauvignon and Cinsault were found to be poor in monomeric and dimeric flavanol content ($p < 0.05$). By contrast to other studies (Guendez, Kallithraka, Makris, & Kefalas, 2005; Rodriguez Montealegre, Romero Peces, Chacon Vozmediano, & Garcia Romero, 2006; Fuleki & da Siva, 1997), the contents were found to be higher since the calculations were based on the freeze-dried matter in this study. Total flavanol content was higher in Alphonso Lavalle and Hamburg Muscat, whereas Cabernet Sauvignon, Cinsault and Merlot varieties have remarkably little amount flavanol content (Table 2).

3.2. Antiradical activity

The results of both DPPH and ORAC tests for the grape seed extracts are listed in Table 3. The EC_{50} (μg/mL) values obtained for the samples submitted to the DPPH assay are in the ranged from 2.71 to 4.62 μg/mL. The lowest EC_{50} values found were for Papaz Karasi and Okuzgozu which were also the richest in polyphenols, whereas Senso exhibited the weakest activity. Merlot and Cabernet Sauvignon, which had lower total polyphenols content, had an important DPPH scavenging activity, but the extract from Hamburg Muscat, with much higher polyphenolic content, had considerably the lower activity. This finding might be ascribed to certain constituents are particularly responsible for strong antioxidant effect (Guendez et al., 2005). The synergic effect of the antioxidants in the extracts should also be considered (Shahidi, Wanasundara, & Amarowicz, 1994; Sun & Ho, 2005).

Oxygen radical absorbance capacities (ORAC) of the grape seed varied from 1425.9 to 3009.2 μmol TE/g seed

Table 2
Monomeric and dimeric flavanol content in grape seeds

	CT	EC	ECG	EGCG	EGC	B1	B2	Galloylated monomers	Total dimer	Total flavanol
Merlot	7.44 ± 0.60^{b}	8.89 ± 0.66^{de}	2.79 ± 0.09^{d}	0.98 ± 0.01^{ab}	2.57 ± 0.18^{b}	0.84 ± 0.06^{bc}	0.72 ± 0.04^{cd}	6.33 ± 0.26^{c}	1.57 ± 0.10^{cd}	24.2 ± 1.62^{b}
Cabernet Sauvignon	9.56 ± 0.53^{c}	7.38 ± 0.40^{c}	0.75 ± 0.02^{ab}	1.31 ± 0.10^{cd}	2.43 ± 0.21^{b}	0.84 ± 0.08^{bc}	0.93 ± 0.07^{ef}	4.48 ± 0.34^{ab}	1.77 ± 0.15^{d}	23.2 ± 1.42^{b}
Cinsoult	11.4 ± 0.42^{d}	5.59 ± 0.21^{b}	2.05 ± 0.07^{cd}	0.79 ± 0.02^{a}	2.53 ± 0.12^{b}	0.75 ± 0.02^{b}	0.66 ± 0.02^{bc}	5.37 ± 0.22^{bc}	1.41 ± 0.04^{bc}	23.8 ± 0.89^{b}
Papaz Karasi	9.28 ± 0.18^{c}	12.3 ± 0.32^{f}	4.71 ± 0.14^{e}	1.15 ± 0.03^{bc}	5.94 ± 0.16^{d}	0.96 ± 0.00^{cd}	0.80 ± 0.02^{de}	11.8 ± 0.32^{e}	1.76 ± 0.02^{d}	35.1 ± 0.84^{e}
Ada Karasi	9.54 ± 0.76^{c}	5.77 ± 0.40^{b}	5.06 ± 0.59^{e}	0.92 ± 0.10^{a}	4.34 ± 0.50^{c}	0.74 ± 0.12^{b}	0.52 ± 0.05^{ab}	10.3 ± 1.20^{de}	1.26 ± 0.17^{b}	26.9 ± 2.54^{bc}
Hamburg Muscat	11.5 ± 0.37^{d}	9.44 ± 0.36^{e}	1.49 ± 0.21^{bc}	2.09 ± 0.12^{e}	2.64 ± 0.18^{b}	1.53 ± 0.08^{e}	1.60 ± 0.08^{h}	6.23 ± 0.50^{c}	3.12 ± 0.16^{f}	30.3 ± 1.39^{cd}
Alphonso Lavallee	12.3 ± 1.05^{d}	12.4 ± 1.08^{f}	2.60 ± 0.20^{d}	1.27 ± 0.08^{cd}	2.41 ± 0.19^{b}	0.99 ± 0.07^{cd}	1.15 ± 0.11^{g}	6.28 ± 0.46^{c}	2.15 ± 0.18^{e}	33.2 ± 2.76^{de}
Okuzgozu	25.8 ± 1.84^{e}	12.4 ± 0.60^{f}	11.5 ± 1.11^{f}	1.42 ± 0.17^{d}	4.23 ± 0.44^{c}	1.11 ± 0.12^{d}	0.72 ± 0.09^{cd}	17.2 ± 1.72^{f}	1.83 ± 0.21^{d}	57.2 ± 4.37^{g}
Bogazkere	4.71 ± 0.12^{a}	2.49 ± 0.01^{a}	0.32 ± 0.07^{a}	0.94 ± 0.01^{a}	1.64 ± 0.03^{a}	0.56 ± 0.01^{a}	0.41 ± 0.01^{a}	2.89 ± 0.11^{a}	0.97 ± 0.02^{a}	11.1 ± 0.00^{a}
Senso	4.96 ± 0.74^{a}	16.88 ± 0.36^{d}	0.53 ± 0.07^{b}	1.33 ± 0.30^{f}	0.79 ± 0.11^{a}	0.56 ± 0.06^{a}	1.38 ± 0.17^{h}	2.66 ± 0.03^{a}	1.93 ± 0.02^{ef}	26.42 ± 1.20^{bc}
Kalecik Karasi	23.8 ± 0.16^{f}	7.76 ± 0.10^{cd}	0.75 ± 0.00^{ab}	2.55 ± 0.03^{f}	5.96 ± 0.02^{d}	1.94 ± 0.01^{f}	0.98 ± 0.02^{f}	9.26 ± 0.02^{d}	2.92 ± 0.01^{f}	43.8 ± 0.28^{f}
Mean	11.84	9.21	2.96	1.34	3.01	0.98	0.90	7.52	1.88	29.1

Values are means ± SD of four assessments and expressed as mg/g seed. Different letters (a–h) within the column show significant differences at $p < 0.05$.
CT: (+)-catechin, EC: (−)-epicatechin, ECG: (−)-epicatechin gallate, EGCG: (−)-epigallocatechin gallate, EGC: (−)-epigallocatechin, B1: Procyanidin B1, B2: Procyanidin B2.

Table 3
DPPH free radical scavenging activities and ORAC values of grape seeds

Varieties	DPPH EC_{50} (μg/ml)	$ORAC_{FL}$ (μmol TE/g dried seed)
Merlot	3.05 ± 0.2^{ab}	2046.7 ± 181.6^{cd}
Cabernet	2.93 ± 0.4^{ab}	1973.2 ± 90.1^{c}
Cinsault	3.55 ± 0.5^{cd}	1644.7 ± 112.7^{b}
Papaz Karasi	2.71 ± 0.2^{a}	2584.8 ± 246.1^{e}
Ada Karasi	2.74 ± 0.1^{a}	2000.6 ± 158.3^{d}
Hamburg Muscat	3.96 ± 0.4^{e}	2225.2 ± 197.2^{d}
Alphonso Lavallee	3.22 ± 0.4^{bc}	1897.8 ± 101.1^{c}
Okuzgozu	2.89 ± 0.1^{ab}	3009.2 ± 129.4^{f}
Bogazkere	3.64 ± 0.2^{de}	1425.9 ± 46.8^{a}
Senso	4.62 ± 0.2^{f}	1627.6 ± 81.6^{ab}
Kalecik Karasi	3.15 ± 0.2^{b}	2198.4 ± 137.3^{de}

Values are means ± SD of four assessments.
Different letters (a–f) within the column show significant differences at $p < 0.05$.

(Table 3). All samples showed good antioxidant properties in terms of in vitro peroxyl radical scavenging activity. The highest ORAC value was found in the Okuzgozu (3009.2 μmol TE/g seed) followed by Papaz Karasi (2584.8 μmol TE/g seed), whereas the lowest one was in Bogazkere (1425.9 μmol TE/g seed). All international varieties had moderate ORAC values compared to some native varieties.

From the Table 3, it was seen that different varieties possess varying degrees of antioxidant potential in two assays. To determine the possible correlation between polyphenol composition and the respective antioxidant activities of the grape seed extracts, linear regression analysis was performed. No significant correlation ($p > 0.05$) was found between neither individual flavanols nor total polyphenols and ORAC values in the seed extracts ($n = 11$). This finding was opposite from some studies reported previously (Guendez et al., 2005; Monagas et al., 2005). Only positive correlation ($r = 0.848$, $p = 0.001$) was found between total flavanols and antioxidant activity of extracts determined by DPPH test. No correlation was observed between individual flavanols by HPLC and DPPH ($p > 0.05$). Several authors have also reported significant correlation between DPPH scavenging activity and the total polyphenol content of a number of grape seed extracts from different varieties (Bakkalbasi et al., 2005; Guendez et al., 2005).

The results obtained the study showed that large differences were found among the varieties in relation to the polyphenol content. Okuzgozu and Papaz Karasi varieties were richest in both total and individual flavanol content among the varieties studied and also showed highest antioxidant activity. In terms of polyphenolic content and antioxidant activity, all native varieties except Bogazkere and Senso had higher values than those of international varieties. All seeds, except Alphonso Lavallee (table grape), examined in this study, have been utilized for wine making. With higher amount of total phenolic content and antioxidant activity, wine making wastes of varieties especially Okuzgozu, Papaz Karasi, Ada Karasi and Kalecik Karasi could be further evaluated as dietary supplement.

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