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Antioxidant Properties and Phenolic Components of Grape Seeds

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ABSTRACT

Grape seeds are primary by-products from grape processing industries. Evaluation of beneficial effects of grape seed components is critical for developing their value-added utilization for improving human health. This review focuses on the antioxidant components and antioxidant properties of grape seeds, as well as the effects of genotype, growing conditions, and post-harvest treatments on antioxidant availability in grape seeds. Also included is a brief summary of factors influencing antioxidant property estimation, along with other health beneficial activities and functionality of grape seed antioxidants. The information may be useful for developing grape seed-derived nutraceuticals and functional food ingredients such as natural antioxidants.

Keywords: anthocyanin, antioxidant, catechin, flavanol, free radical, grape seed, phenolic, resveratrol

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INTRODUCTION

Grapes are one of the largest fruit crops in the world, with approximately 66 million tons produced world wide in 2007, and over 6.1 million tons produced in the United States alone (http://faostat.fao.org/site/567/default.aspx#ancor). Approximately 86.6% of fresh grapes are processed to produce wine, jams, and grape juice (Maier *et al.* 2009). Seeds compose 5% by-mass of grapes and are a primary by-product from grape processing industries. Grape seeds are composed of 10-20% oil, along with fiber, protein, and other components including phenolic antioxidants (Kim *et al.* 2006; Choi and Lee 2008). Investigation of health beneficial properties such as antioxidative capacity is important for development of value-added utilization of grape seeds (Parry *et al*. 2006). A number of studies have investigated antioxidant components of grape seeds and seed fractions (Luther *et al.* 2007). Antioxidant properties of grape seeds, seed fractions, and individual grape antioxidant compounds have also been evaluated. This review summarizes the available information on phenolic components in grape seeds, their antioxidant properties, effects of post-harvest treatments on grape antioxidants, and considerations for grape antioxidant property estimation.

PHENOLIC COMPONENTS

Total phenolic content

Grape seeds are rich in phenolic compounds such as anthocyanin, the glucosides of anthocyanidins (**Fig. 1A**), catechin (**Fig. 1B**), and gallic acid (**Fig. 1C**). Polyphenolics generally contain two or more hydroxyl groups attached to a conjugated ring system such as a benzene ring. Polyphenols contribute to the overall antioxidant properties of grape and may have important health benefits, including possible preventative effects against cancer (Fan and Lou 2004) and cardiovascular diseases (Zern *et al.* 2003; Zern *et al.* 2005). Anthocyanins have been shown to protect against lipid peroxidation and DNA damage in rat hepatoma cells *in vitro*, demonstrating potential anticarcinogenic properties (Lazzé *et al.* 2003). Flavanols, such as catechin, have demonstrated inhibitory effects on platelet reactivity *in vitro*, a property which may reduce the risk of cardiovascular disease (Pearson *et al.* 2005). Gallic acid has been shown to exhibit selective cytotoxicity *in vivo* against a variety of human and mouse cancer cells (Inoue *et al.* 1995).

Table 1 summarizes the total phenolic (TPC), total flavanol (TFC), and total anthocyanin (TAC) contents of sel-

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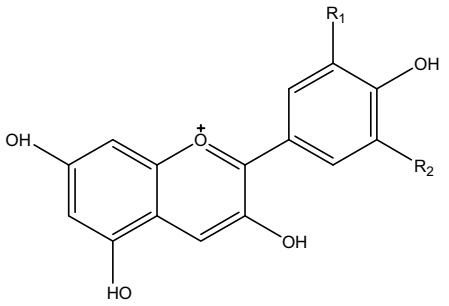
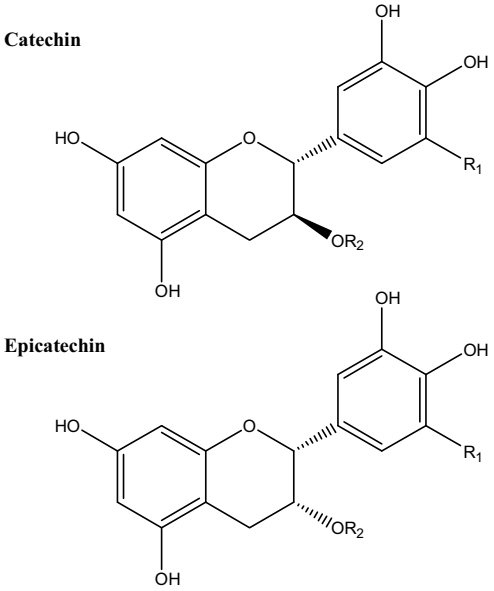
Invited Review



Anthocyanidin	R1	R2
Cyanidin	OH	H
Delphinidin	OH	OH
Malvidin	OMe	OMe
Pelargonidin	H	H
Petunidin	OH	OMe
Peonidin	OMe	H



Catechin	Epicatechin	R1	R2
(+)-Catechin	(-)-Epicatechin	H	H
(+)-Gallocatechin	(-)-Epigallocatechin	OH	H
(+)-Catechin-3-gallate	(-)-Epicatechin-3-gallate	H	Gallate
(+)-Gallocatechin-3-gallate	(-)-Epigallocatechin-3-gallate	OH	Gallate

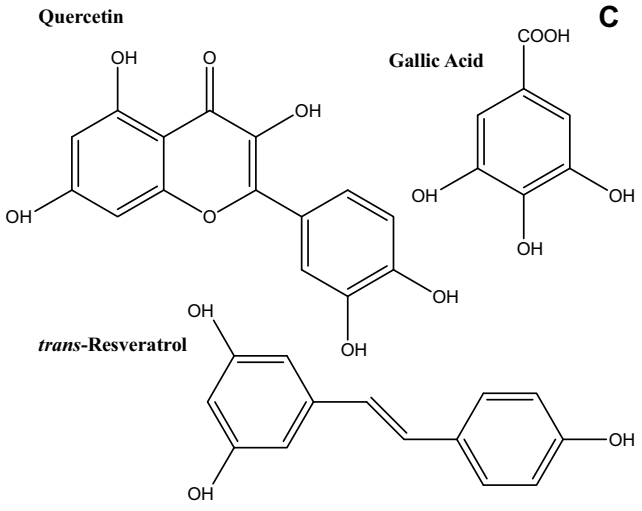


Fig. 1 Chemical structures of grape phenolic compounds. (**A**) Anthocyanidins; (**B**) Catechins (structure on the left is a (+)-catechin skeleton, while the structure on the right is an (-)-epicatechin skeleton); and (**C**) Quercetin, gallic acid, and *trans*-resveratrol.

ected grape cultivars estimated using spectrophotometric methods from previous studies. In general, TPC values were determined colorimetrically using Folin-Ciocalteu (FC) reagent, which may also react with other non-phenolic reducing agents, such as ascorbic acid. Of the grape varieties analyzed, Papaz Karasi seeds contained the highest total phenolic content of 154.6 mg gallic acid equivalents (GAE) /g on a per dry seed weight basis, followed by Okuzgozu at 139.4 mg GAE/g, and Ada Karasi at 137.5 mg GAE/g (Bozan *et al.* 2008). Of the grape seeds reported on a per fresh weight basis, the seeds of Muscat of Alexandria contained the highest total phenolic content of 54.9 mg GAE/g seed (Poudel *et al.* 2008), followed by 32.6 mg GAE/g in Summit Muscadine (bronze) and 26.9 mg GAE/g in Noble Muscadine (purple) grape seeds (Pastrana-Bonilla *et al.* 2003). Under the same experimental conditions, the pulp, skin, and whole fruit of Summit Muscadine grape (**Table 2**) contained about 0.22, 5.41, and 3.10 mg GAE/g on a fresh weight basis, respectively, which is less than 1, 20, and 10% of that in the seeds (Pastrana-Bonilla *et al.* 2003). Pastrana-Bonilla *et al.* (2003) analyzed the TPC of 10 different varieties of Muscadine grapes, five with bronze skins and five with purple skins. The seeds of the five bronze grapes had TPC value of 19.2-32.6 mg GAE/g, which was much higher than that of 3.0-5.5, 0.21-0.25, and 1.7-3.1 mg GAE/g determined in the skin, pulp, and whole fruit, respectively (**Table 2**). The total phenolics in the seeds were, on average, five times more concentrated than that in the skin and 80 times more concentrated than that in the pulp on a fresh weight basis (**Table 2**), suggesting that grape seeds may serve as an excellent source for dietary phenolic components.

In addition, the TPC of grape seed flours, oils, and extracts have been investigated. In 2006, Parry and others (Parry *et al.* 2006) reported the TPC value of various fruit seed flours, including that of Pinot Noir and Chardonnay grape varieties. Chardonnay seed flour exhibited the highest TPC with 186.3 mg GAE/g flour, higher than that of Pinot Noir, and also higher than that of black and red raspberry, blueberry, and cranberry seed flours (Parry *et al.* 2006). Bail *et al.* (2008) analyzed the TPC values of nine European grape seed oils, and found that the highest TPC value was 0.1 mg GAE/g seed oil whereas the lowest was 0.06 mg GAE/g oil, suggesting that grape seed oils may not be a good source of phenolic compounds (Bail *et al.* 2008). TPC of seed extracts of several Turkish grape varieties were evaluated by Yemis *et al*. (2008), and Narince grape seed extract was shown to contain the highest total phenolic content with 587.3 mg GAE/g extract, while the lowest TPC value was 339.5 mg GAE/g extract (Yemis *et al.* 2008). In 2009, using HPLC, Maier *et al*. determined TPC of the intact grape seeds, seed oil press residues, and the seed flour which is the byproduct of grape seed-oil extraction of seven cultivars of *Vitis vinifera* L. The seeds had TPC values ranging from 188.7 to 1165.8 mg/kg dry matter (DM), which was statistically higher than the corresponding values for the seed flour, ranging from 147.4 to 492.7 mg/kg DM, suggesting that grape seed oil may contain significant levels of phenolic compounds, while the seed flour may also serve as a dietary source of phenolics (Maier *et al.* 2009).

Highest reported total flavanol content (TFC) per fresh weight was found in Shiohtashibudou grape seeds, at a level of 5.5 mg quercetin equivalents (QE)/g (Poudel *et al.* 2008), while Papaz Karasi contained the highest flavanol content on per dry seed weight basis, with 179.4 catechin equivalents (CE)/g (Bozan *et al.* 2008). **Table 1** provides a summary of representative TFC values of grape seeds from several previous studies. The seeds, skins, and pulp of Pinot Noir, Pinot Meunier, and the Chardonnay grape were analyzed with HPLC (Mané *et al.* 2007). The results showed that seed of the Pinot Noir, Pinot Meunier, and Chardonnay grapes had TFC values of 75.1, 101, and 57.6 mg/g, respectively, on a per fresh weight basis, which were much greater than that of 30, 24, and 21 mg/g detected in the corresponding skin fractions, or that of 0.45, 0.26, and 0.36 mg/g in

Table 1 Total phenolic, flavanol, and anthocyanin contents of grape seeds.

Cultivar	TPC (mg GAE/g)	TFC (mg/g)	TAC (mg CGE/100 g)	Reference
Ebizuru	8.8 [F]	1.3 QE [F]	ND	Poudel *et al.* 2008
Ryukyuganebu	3.6 [F]	1.0 QE [F]	ND	Poudel *et al.* 2008
Shiohtashibudou	13.6 [F]	5.5 QE [F]	ND	Poudel *et al.* 2008
Shiragabudou	16.5 [F]	1.4 QE [F]	ND	Poudel *et al.* 2008
Yamabudou	5.7 [F]	0.8 QE [F]	ND	Poudel *et al.* 2008
Kadainou R-1	8.7 [F]	0.9 QE [F]	ND	Poudel *et al.* 2008
Kadainou R-1 x Bailey Alicante A	8.7 [F]	0.7 QE [F]	ND	Poudel *et al.* 2008
Bailey Alicante A	17.9 [F]	0.9 QE [F]	ND	Poudel *et al.* 2008
Muscat of Alexandria	54.9 [F]	1.0 QE [F]	ND	Poudel *et al.* 2008
Merlot	105.7 [D]	122.7 CE [D]	NA	Bozan *et al.* 2008
Cabernet	103.7 [D]	125.0 CE [D]	NA	Bozan *et al.* 2008
Cinsault	88.1 [D]	97.1 CE [D]	NA	Bozan *et al.* 2008
Papaz Karasi	154.6 [D]	179.4 CE [D]	NA	Bozan *et al.* 2008
Ada Karasi	137.5 [D]	163.4 CE [D]	NA	Bozan *et al.* 2008
Hamburg Muscat	104.4 [D]	105.7 CE [D]	NA	Bozan *et al.* 2008
Alphonso Lavallee	105.3 [D]	123.3 CE [D]	NA	Bozan *et al.* 2008
Okuzgozu	139.4 [D]	174.5 CE [D]	NA	Bozan *et al.* 2008
Bogazkere	94.2 [D]	95.0 CE [D]	NA	Bozan *et al.* 2008
Senso	79.2 [D]	89.2 CE [D]	NA	Bozan *et al.* 2008
Kalecik Karasi	136.2 [D]	147.7 CE [D]	NA	Bozan *et al.* 2008
Bronze Muscadine	19.2-32.6[a F]	NA	1.2-8.7[a F]	Pastrana-Bonilla *et al.* 2003
Purple Muscadine	15.4-26.9[b F]	NA	2.2-7.5[b F]	Pastrana-Bonilla *et al.* 2003
Cabernet Sauvignon	8.7 [F]	NA	NA	Guendez *et al.* 2005
Grenache Rouge	9.8 [F]	NA	NA	Guendez *et al.* 2005
Merlot	16.9 [F]	NA	NA	Guendez *et al.* 2005
Mandilaria	22.3 [F]	NA	NA	Guendez *et al.* 2005
Agiorgitiko	11.3 [F]	NA	NA	Guendez *et al.* 2005
Negoska	11.8 [F]	NA	NA	Guendez *et al.* 2005
Xinomavro	1.4 [F]	NA	NA	Guendez *et al.* 2005
Mavrodafni	4.0 [F]	NA	NA	Guendez *et al.* 2005
Limnio	14.1 [F]	NA	NA	Guendez *et al.* 2005

[a]: range for five different cultivars of bronze Muscadine grapes; [b]: range for five different purple Muscadine grapes.
TPC: total phenolic content; TFC: total flavanol content; TAC: total anthocyanin content; GAE: gallic acid equivalent; QE: quercetin equivalent; CE: catechin equivalent; CGE: cyanidin-3-glucoside equivalent; [D]: dry weight; [F]: fresh weight; NA: not analyzed; ND: not detected.

Table 2 Total phenolic content, anthocyanin content, and antioxidant capacity of whole fruit and parts of Muscadine grapes.[a]

Cultivar	TPC (mg GAE/100 g)				TAC (mg CGE/100 g)				TEAC (μM/g)			
	Seed	Skin	Pulp	Whole fruit	Seed	Skin	Pulp	Whole fruit	Seed	Skin	Pulp	Whole fruit
Bronze Varieties												
Bronze Carlos	1920.3	545.6	25.1	307.9	1.2	2.6	ND	0.9	204.6	14.9	3.4	18.2
Early Fry	2367.2	303.0	21.3	169.1	8.7	2.5	ND	1.1	277.8	13.9	2.0	11.2
Fry	2356.3	332.2	23.8	199.0	4.6	0.8	ND	0.4	234.2	11.1	2.9	9.8
Summit	3258.7	541.0	22.3	309.7	3.1	2.8	ND	1.3	245.4	12.4	3.0	10.2
Late Fry	1986.0	348.9	24.0	252.3	3.7	2.0	ND	1.1	218.9	13.4	2.4	15.4
AV	2377.7	414.1	23.3	247.6	4.3	2.1	ND	1.0	236.2	13.1	2.7	13.0
Purple Varieties												
Purple Paulk	1649.3	363.6	30.0	195.2	4.1	177.0	4.7	74.8	307.9	12.1	2.2	11.2
Cowart	2303.0	261.6	11.6	214.2	4.6	107.8	1.1	37.8	325.5	12.4	2.7	21.7
Supreme	1535.5	329.9	20.1	184.7	7.5	135.5	0.7	65.2	478.6	12.2	1.6	11.5
Ison	1726.2	365.0	26.0	218.9	4.6	174.5	1.9	69.5	284.9	13.3	2.1	15.9
Noble	2685.3	355.1	33.4	425.7	2.2	65.5	2.2	31.5	234.7	12.4	2.1	27.8
AV	1979.9	335.0	24.2	247.7	4.6	132.1	2.1	55.8	326.3	12.5	2.1	17.6

[a] Data from Pastrana-Bonilla *et al.* 2003
TPC: total phenolic content; TAC: Total anthocyanin content; TEAC: trolox equivalent antioxidant capacity; GAE: gallic acid equivalent; CGE: cyanidin 3-glucoside equivalent; ND: not detected; AV: average value

the corresponding pulp samples (Mané *et al.* 2007). It needs to be pointed out that these TFC values might be estimated using catechin, epicatechin, epigallocatechin, or epicatechin-3-gallate as a standard compound (Mané *et al.* 2007). In addition, Maier *et al.* (2009) compared the TFC of seven grape seeds and their corresponding seed flours, the residue from seed oil press, and showed that the seeds contained 4.39-18.78 g TFC/kg and the seed flours had 2.5-13.5 g TFC/kg on per dry mass basis. The seed flour retained about 57-78.6% TFC after seed oil production, indicating that both whole seeds and seed flour, the by-product from seed oil preparation, may serve as a dietary source of flavonoids.

Total anthocyanin content (TAC) has been quantified for grape seeds in a number of previous studies using a UV-visible spectrophotometer according to a pH-differential method. Highest reported total anthocyanin content (TAC) was found in Early Fry Muscadine (bronze) grape seeds (**Table 2**), with 8.7 mg cyanidin-3-glucoside equivalents (CGE)/100 g fresh seeds (Pastrana-Bonilla *et al.* 2003). It was interesting that seeds of bronze Muscadine grapes had TAC value ranging from 1.2 to 8.7 mg CGE/100 g fresh seeds, whereas that of purple grapes had a TAC range of 2.2-7.5 mg CGE/100 g fresh seeds (Pastrana-Bonilla *et al.* 2003), suggesting that that TAC values of grape seeds could not predicted by the pigmentation in grape skins, although anthocyanin is the pigment responsible for the purple color in both grapes and wine. The data, in **Table 2**, show that

there were low levels of anthocyanins in the seed and no anthocyanins detected in the pulp of the bronze cultivars of Muscadine grapes. The anthocyanin content was only slightly higher in the skins of these varieties on a fresh weight basis (**Table 2**). In the purple-skinned cultivars of Muscadine grapes, the seeds and pulp contained lower levels of anthocyanin at 2.2-7.5 and 0.7-4.7 mg CGE/100 g respectively, but the skins had high concentration ranging from 65.5 to 177.0 mg CGE/100 g on a per fresh sample weight basis (Pastrana-Bonilla *et al.* 2003). Total anthocyanin content in the seeds of purple Muscadines grapes was, on average, 1.3 times higher than that of the bronze grapes, while the skins of purple cultivars had about 65 times more anthocyanins than that of bronze varieties (Pastrana-Bonilla *et al.* 2003).

In summary, TPC values vary greatly in grape seeds and seed fractions, suggesting that genotype and environmental conditions during growth may alter the availability of phenolic components in grape seeds. However, overall, grape seeds appear to be an excellent source of phenolic compounds, and can contribute significantly to the economic value of grapes for the viticulture industry.

Phenolic composition of grape seeds

In addition to TPC, the quantities of individual polyphenolic compounds have also been of interest to researchers because they may contribute to the overall and selected health beneficial effects differently. For example, they may have different antioxidant properties. Catechins, anthocyanins, and other phenolic compounds such as gallic acid have been detected in grape seeds (**Fig. 1**) (Fuleki and Ricardo da Silva 1997; Kammerer *et al*. 2004; Guendez *et al*. 2005; Montealegre *et al*. 2006; Maier *et al*. 2009). **Table 3** shows the concentrations of major polyphenolic compounds reported in grape seeds; only a small part of the values from previous studies were included. Of the varieties reported on a dry weight basis, the highest concentration of catechin was found in the seeds of Okuzgozu cultivar at 2580 mg/100 g, and the highest epicatechin concentration was found to be 1688 mg/100 g dry mass in Senso seeds (Bozan *et al.* 2008). Okuzgozu and Kalecik Karasi grape seeds had the highest epicatechin gallate (1150 mg/100 g) and epigallocatechin gallate (255 mg/100 g) contents, respectively (Bozan *et al.* 2008), while the highest proanthocyanidin B1 and B2 content was observed in Spätburgunder grape seeds measuring up to 499 mg/100 g and 298 mg/100 g, respectively (Maier *et al.* 2009). Of the varieties measured on a per fresh weight basis, the most remarkable one was the seeds of Mandilaria cultivar that had the highest levels of catechin at 454 mg/100 g, epicatechin at 249 mg/100 g, epicatechin gallate at 64.4 mg/100 g, epigallocatechin gallate at 15.6 mg/100 g, procyanidin B1 at 102 mg/100 g, and procyanidin B2 at 69.2 mg/100 g, along with the greatest total polyphenolic concentration and the second highest concentration of gallic acid at 10.5 mg/100 g (Guendez *et al.* 2005). Catechin and epicatechin were, on average, the most abundant polyphenolic compounds in the seeds of the analyzed grape varieties. Of the rest of the compounds analyzed, proanthocyanidin B2 and epicatechin gallate were the next most abundant, depending on the variety of grape and method of estimation.

Grape parts also may differ in the concentrations of individual phenolic compounds (Monagas *et al.* 2003; Kammerer *et al.* 2004; Yilmaz and Toledo 2004; Montealegre *et al.* 2006; Iacopini *et al.* 2008; Huang *et al.* 2009). **Table 4** compares the levels of catechin, epicatechin, and procyanidin B1 in the seeds and skins of eleven grape varieties including four red and six white. The tested seeds exhibited higher levels of catechin, epicatechin, and procyanidin B1 than their corresponding skin samples on a per sample weight basis regardless of grape skin color (**Table 4**). This observation is supported by the results from a number of other studies (Monagas *et al.* 2003; Yilmaz and Toledo 2004). In 2003, Monagas *et al*. examined the levels of anthocyanins and flavanols in the skin, seeds, and wine of Tempranillo, Graciano, and Cabernet Sauvignon grapes. Phenolic compositions differed greatly between the skin and seeds of same grapes and between seeds from different grape samples (Monagas *et al.* 2003). The seeds had higher levels of all detected flavanol and anthocyanin compounds than the corresponding skin samples. For instance, Tempranillo seeds contained (-)-epicatechin at a level of 0.62 mg/g, whereas the skin had a (-)-epicatechin concentration of 0.079 mg/g on a dry weight basis (Monagas *et al.* 2003). Another study by Yilmaz and Toledo also detected higher levels of catechin in the seeds as opposed to the skins of Chardonnay and Merlot grapes (Yilmaz and Toledo 2004). It was also reported from this study that seeds of both cultivars had greater gallic acid concentrations (15 and 10 mg/100 g dry seeds compared to 5 and 3 mg/100 g dry skin, respectively) for Chardonnay and Merlot grapes. It needs to be pointed out that a few studies reported the levels of quercetin, resveratrol, rutin, myricetin, cyaniding glucoside and other phenolic compounds in grape skins, but did not report their presence in the seeds (Pastrana-Bonilla *et al.* 2003; Iacopini *et al*. 2008; Huang *et al*. 2009). This indicated that grape seeds may have unique phenolic composition compared to the skin parts and could be utilized for different beneficial effects.

Table 3 Polyphenolic composition of grape seeds.

Cultivar	CT	EC	ECG	EGCG	B1	B2	GA
Cabernet Sauvignon [a]	215 [F]	89.3 [F]	27.9 [F]	6.5 [F]	14.8 [F]	11.3 [F]	2.8 [F]
Grenache Rouge [a]	203 [F]	86.8 [F]	18.6 [F]	9.5 [F]	10.6 [F]	6.1 [F]	3.4 [F]
Merlot [a]	183 [F]	83.4 [F]	58 [F]	13.5 [F]	13.5 [F]	17.6 [F]	2.7 [F]
Mandilaria [a]	454 [F]	249 [F]	64.4 [F]	15.6 [F]	102 [F]	69.2 [F]	10.5 [F]
Agiorgitiko [a]	245 [F]	172 [F]	41.3 [F]	10.9 [F]	31.9 [F]	36.1 [F]	17.9 [F]
Xinomavro [a]	36.7 [F]	17.5 [F]	0.1 [F]	0.1 [F]	0 [F]	0.1 [F]	0.7 [F]
Limnio [a]	51.3 [F]	20.1 [F]	13.8 [F]	0.3 [F]	0 [F]	0.1 [F]	1.2 [F]
Turkish Varieties [b]	471-2580 [D]	249-1688 [D]	32-1150 [D]	79-255 [D]	56-194 [D]	41-160 [D]	NA
Spätburgunder [c]	376 [D]	612 [D]	92 [D]	NA	499 [D]	298 [D]	NA
Samtrot [c]	464 [D]	331 [D]	198 [D]	NA	207 [D]	273 [D]	NA
Müller-Thurgau [c]	217 [D]	206 [D]	80 [D]	NA	121 [D]	121 [D]	NA
Kerner	88 [D]	223 [D]	41 [D]	NA	61 [D]	116 [D]	NA
Schwarzriesling [c]	238 [D]	266 [D]	169 [D]	NA	91 [D]	122 [D]	NA
White grape [d]	12-50 [D]	11-31 [D]	1.3-6.7 [D]	NA	20-62 [D]	1.9-3.3 [D]	NA
Red grape [d]	8.2-27 [D]	6-21 [D]	3.2-7 [D]	NA	7.4-17 [D]	2.1-4.1 [D]	0.7-1.0 [D]
Vinifera [e]	25-244 [D]	24-193 [D]	NA	NA	11-62 [D]	29-93 [D]	NA
Hybrid [e]	21-155 [A]	23-284 [A]	NA	NA	3-60 [A]	9-106 [A]	NA
Labruska [e]	37-58 [A]	55-97 [A]	NA	NA	7-11 [A]	29-75 [A]	NA
Weisser Riesling [f]	79 [D]	67.5 [D]	45.8 [D]	NA	105.4 [D]	50.6 [D]	NA

References: [a] - Guendez *et al.* 2005; [b] - Bozan *et al.* 2008; [c] - Maier *et al.* 2009; [d] - Montealegre *et al.* 2006; [e] - Fuleki and Ricardo da Silva 1997; [f] - Kammerer *et al.* 2005.
All quantities expressed in mg/100 g seed. CT: (+)-catechin; EC: (-)-epicatechin; ECG: (-)-epicatechin gallate; EGCG: (-)-epigallocatechin gallate; B1: proanthocyanidin B1; B2: proanthocyanidin B2; GA: gallic acid; [D]: dry weight; [F]: fresh weight; [A]: air dried; NA: not analyzed.

Table 4 Polyphenolic composition of seeds and skins of red and white grape varieties.

cultivar	Catechin		Epicatechin		Procyanidin B1	
	Seed	Skin	Seed	Skin	Seed	Skin
Weisser Riesling 2002 [a](White)	79.0 [D]	22.7 [D]	67.5 [D]	13.5 [D]	105.4 [D]	19.2 [D]
Riesling [b] (White)	40.0 [F]	1.4 [F]	16.0 [F]	trace	62.0 [F]	1.2 [F]
Merlot [b] (Red)	24.0 [F]	2.5 [F]	21.0 [F]	1.3 [F]	17.0 [F]	2.1 [F]
Cabernet Sauvignon [b] (Red)	27.0 [F]	1.7 [F]	13.0 [F]	0.6 [F]	15.0 [F]	1.2 [F]
Chardonnay [b] (White)	39.0 [F]	2.3 [F]	31.0 [F]	0.6 [F]	38.0 [F]	2.3 [F]
Sauvignon Blanc [b] (White)	20.0 [F]	1.0 [F]	13.0 [F]	0.3 [F]	25.0 [F]	1.6 [F]
Moscatel [b] (Red)	35.0 [F]	1.6 [F]	12.0 [F]	0.3 [F]	33.0 [F]	2.1 [F]
Gewürztraminer [b] (White)	50.0 [F]	1.9 [F]	15.0 [F]	0.8 [F]	46.0 [F]	4.8 [F]
Viogner [b] (White)	12.0 [F]	trace	11.0 [F]	trace	20.0 [F]	trace
Cencibel [b] (Red)	8.2 [F]	2.2 [F]	6.0 [F]	0.8 [F]	7.4 [F]	2.2 [F]
Shiraz [b] (Red)	12.0 [F]	0.9 [F]	13.0 [F]	0.7 [F]	10.0 [F]	0.8 [F]

References: [a] - Montealegre *et al.* 2006; [b] - Kammerer *et al.* 2005.
All quantities expressed in mg/100 g seed. [D]: dry weight; [F]: fresh weight. White: white wine; Red: red wine.

Trans-Resveratrol

Trans-Resveratrol (*trans*-3,5,4'-trihydroxystilbene, sometimes shortened to resveratrol) is a polyphenolic compound naturally present in grape skins and seeds (**Fig. 1C**). *Trans*-resveratrol has been shown to have a number of health beneficial effects including inhibition of platelet aggregation, anti-inflammatory activity, antioxidant properties, capacity to reduce the risk of cancer and cardiovascular disease, and possible longevity promoting effect (Howitz *et al.* 2003; Wood *et al*. 2004; Yilmaz and Toledo 2004; Li *et al.* 2006; Iacopini *et al.* 2008). The levels of *trans*-resveratrol in grape seeds and skin were investigated (Li *et al.* 2006; Iacopini *et al.* 2008). Extractable or available amounts of *trans*-resveratrol in the seeds and skin of 120 grape cultivars grown in two years have been compared (Li *et al.* 2006). Methanol at a solvent-solid ratio of 5 mL for each gram of frozen seeds was used for *trans*-resveratrol extraction at 25°C for 48 hours in dark. Results from this study showed that grape seeds contained significant level of *trans*-resveratrol (Li *et al*. 2006). This study also showed that both cultivar type and growing conditions altered its level in seeds and skin. The level of *trans*-resveratrol varied from 1.06 to 17.03 μg/g in the tested grape seeds, and varied from 0.56 to 145.11 μg/g skin on per fresh weight (Li *et al.* 2006). Importantly, seeds of some cultivars of grape contained greater level of *trans*-resveratrol than their counterpart skin samples. Taken together, these data indicated that grape seeds and skin may serve as dietary sources for *trans*-resveratrol.

In contrast, a recent study detected no *trans*-resveratrol in the grape seeds, while significant level of *trans*-resveratrol was found in grape skin samples under the same experimental conditions (Iacopini *et al.* 2008). Ethanol: water: hydrochloric acid (0.12 M) at 70:29:1 (v/v/v) was used for extracting resveratrol and the extraction was performed in 4 hours. While the difference in grape cultivars could be a possible explanation for the absence of extracted *trans*-resveratrol from grape seeds, it could also have been partially due to a different extraction method.

ANTIOXIDANT PROPERTIES

Antioxidative properties of grape seeds

Grape seeds have been shown to have antioxidative properties (Jayaprakasha *et al*. 2003; Janisch *et al.* 2006; Kim *et al*. 2006; Yilmaz and Toledo 2006; Bozan *et al.* 2008; Iacopini *et al.* 2008; Poudel *et al*. 2008). The seeds of *V. vinifera* variety Bangalore blue grapes grown in India were analyzed for their capacity to reduce Mo (VI) to Mo (V) and to quench 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals (Jayaprakasha *et al*. 2003). The seed extracts exhibited dose-dependent $DPPH^{\bullet}$ scavenging property and reducing power under the experimental conditions. In 2008, defatted seeds of 11 grape cultivars grown in Turkey were extracted with acetone: water (70: 30, v/v) containing 0.5% acetic acid at 50°C and the extracts were evaluated for their free radical scavenging activities (Bozan *et al.* 2008). All eleven seed extracts showed significant ability to directly react with and quench peroxyl (ORAC) and DPPH radicals (Bozan *et al.* 2008). The greatest ORAC value was 3.0 mmol trolox equivalents (TE)/g dry seeds determined in the Okuzgozu grape seeds, and the lowest ORAC value was 1.4 mmol TE/g for Bogazkere seeds; a 2-fold difference (**Fig. 2**). The DPPH radical scavenging capacity was reported in EC_{50} value, which is the required antioxidant concentration to quench 50% of the radicals in the system under the assay conditions. The Papaz Karasi grape seeds had smallest EC_{50} value against DPPH radicals, which represented the greatest DPPH radical scavenging capacity, whereas the Okuzgozu seeds had an EC_{50} value of 2.89 μg/mL, the third strongest DPPH radical scavenging ability among the eleven grape seeds (Bozan *et al.* 2008). DPPH radical scavenging capacity was also determined for five wild grapes in another study (Poudel *et al*. 2008). Another study showed the $DPPH^{\bullet}$ and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) radical ($ABTS^{\bullet+}$) scavenging capacities of 12 varieties of *V. vinifera* grape grown in Turkey (Yemis *et al*. 2008). All tested grape seeds showed significant scavenging capacity against both $DPPH^{\bullet}$ and $ABTS^{\bullet+}$. While the different grape varieties ranked differently in $ABTS^{\bullet+}$ scavenging and $DPPH^{\bullet}$ scavenging, both $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ scavenging capacities were significantly correlated to the total phenolic contents of the seeds (Yemis *et al*. 2008). In 2009, a study compared grape seed extract with ascorbic acid and chlorhexidine for their ability to directly react with and quench chemically generated ABTS cation radicals (Furiga *et al*. 2009). The grape seed extract had the greatest $ABTS^{\bullet+}$ scavenging capacity under the experimental conditions.

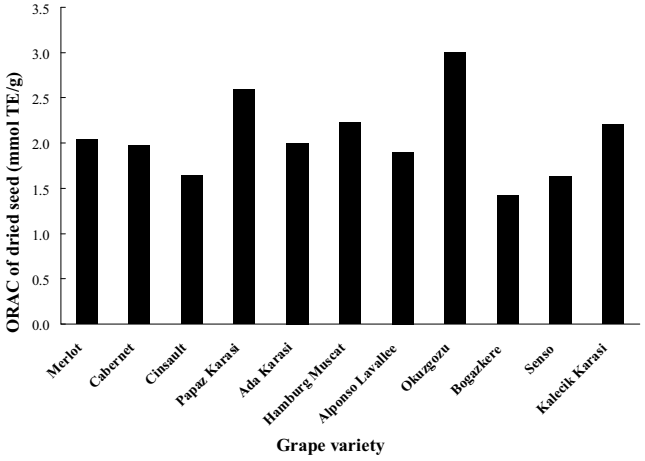


Fig. 2 Oxygen radical absorbance capacity (ORAC) of grape seed extracts. Adapted from Bozan *et al*. (2008).

These results indicated that these grape seeds may differ in the content and compositions of their antioxidative components, which might have interacted with peroxyl (ORAC), ABTS cation, and DPPH radicals in different manners under the assay conditions. It is also widely accepted that individual antioxidant activity assays differ in their determination principles and antioxidant activity estimation depends on the assays selected.

Grape seeds have also been compared with other grape parts for antioxidant properties. The pulp, peel, and seeds of red rose grape from a Chinese local market were compared for their ferric reducing/antioxidant power (FRAP) using $FeSO_4$ as the standard (Guo *et al*. 2003). The seeds had a FRAP value of about 56 mmol/100 g, which was much greater than 0.49 and 11 mmol/100 g for pulp and skin, respectively, on a per fresh weight basis. In 2008, seeds and skins of 10 native Tuscan and international red grape samples were compared for their DPPH radical scavenging abilities (Iacopini *et al.* 2008). The IC_{50} values, which are the required antioxidant concentration to quench 50% of the radicals in the assay mixtures under the experimental conditions, were estimated. The antioxidants were extracted using ethanol: water: hydrochloric acid (0.12 M) (70: 29: 1, v/v/v). The seeds of Sangiovese clone ISV RC1 and the skin of Merlot grapes had the strongest DPPH radical scavenging capacity with the lowest same IC_{50} value of 1.74 mg GAE/L. In 2006, Yilmaz and Toledo compared the peroxyl radical scavenging capacity (ORAC) for the seeds and skin of Merlot and Chardonnay grapes (Yilmaz and Toledo 2006). The seeds had ORAC values of 345 and 638 μmol TE/g for Merlot and Chardonnay grapes, respectively, which were much higher than that of 70 and 103 μmol TE/g for the counterpart skin samples on a per dry weight basis (Yilmaz and Toledo 2006).

Recently, Choi and Lee (2008) reported that a tocotrienol-rich fraction prepared from Campbell early grape seeds had scavenging capacity against $ABTS^{\bullet+}$ and $DPPH^{\bullet}$, Fe^{2+} chelating activity, reducing power, and inhibition of linoleic acid oxidation. These results agreed with the observations from an earlier study showing that Chardonnay grape seed flour extract could suppress overall lipid peroxidation and prevent oxidative loss of longer chain ω-3 polyunsaturated fatty acids in fish oil (Luther *et al*. 2007). The seed flour was the residue from seed oil preparation by cold-pressing. This study also demonstrated peroxyl radical scavenging capacity (ORAC) of Chardonnay seed flours, which was more than 6 times higher than the black raspberry seed flour on a per dry flour weight basis under the same assay conditions. $DPPH^{\bullet}$ scavenging activity of the grape seed extract at a final concentration of 26 mg seed flour equivalent/mL was similar to that of the black raspberry seed extract, and greater than that observed for 50 ppm of the mixed tocopherol (Luther *et al.* 2007). In addition, the cold-pressed Chardonnay and Pinot Noir grape seed flours were demonstrated for their ORAC, $DPPH^{\bullet}$ scavenging, and Fe^{2+} chelating capacities (Parry *et al*. 2006).

Individual grape phenolic compounds have been investigated for their antioxidant properties in human low-density lipoprotein (LDL) (Janisch *et al.* 2006). Reduced degree of LDL oxidation has been associated with a lower plaque formation in arteries (Stocker and Keaney 2004). The required concentration for gallic acid, catechin, epicatechin and procyanidins B1, B2, B3, C1 and EB5 to achieve the maximum lag time measured as diene formation was determined in the study. Procyanidin EB5 was the most effective compound to prevent lipid peroxidation in the LDL under the experimental conditions (**Fig. 3**). The ability of these compounds to quench hydroxyl and peroxyl anion radicals generally followed the order of suppressing LDL oxidation (Janisch, *et al.* 2006). Earlier in 1991, catechin, epicatechin, epicatechin gallate, procyanidins B2 and B5 and C1, procyanidin B2 gallate, as well as procyanidin trimer 2 and trimer 3 showed peroxide anion radical scavenging capacity at pH 7.5 and pH 9.0 conditions (Ricardo da Silva *et al*. 1991). In 2008, individual grape phenolic compounds such as catechin, epicatechin, rutin, *trans*-resveratrol, and quercetin were shown to have scavenging capacity against $DPPH^{\bullet}$ and peroxynitrite (Iacopini *et al.* 2008). In addition, gallic acid and catechin were reported for their scavenging capacity against $DPPH^{\bullet}$, peroxide anion ($O_2^{\bullet-}$) and hydroxyl ($HO^{\bullet}$) radicals, and their reducing power (Spranger *et al*. 2008).

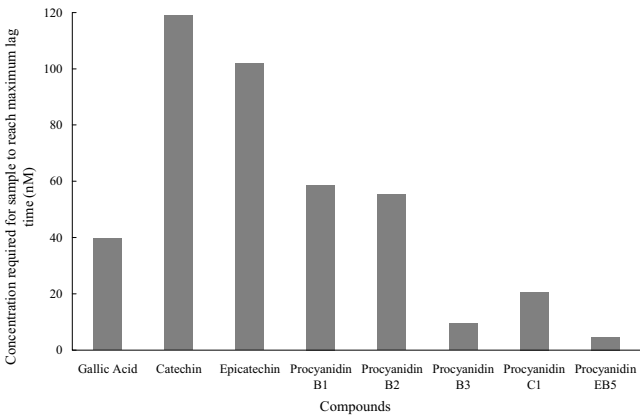


Fig. 3 Inhibitory effects of individual grape phenolic compounds on lipid peroxidation in human LDL. Results were expressed in nanomolar of each phenolic compound required to reach maximum lag time (adapted from Janisch *et. al* 2006). The lag time was determined by photometrically tracking accumulation of conjugated diene formation through absorbance at λ = 234 nm.

Finally, *trans*-resveratrol was compared with other grape phenolic compounds for antioxidant properties. *Trans*-resveratrol exhibited a peroxyl radical scavenging capacity (ORAC value) of 29.06 μmol trolox equivalent (TE)/mg, while catechin had an ORAC value of 20.53 μmol/mg under the same assay conditions, which was followed by epicatechin, gallocatechin, gallic acid, and ellagic acid with a range of ORAC value from 20.53 to 3.88 μmol TE/mg (Yilmaz and Toledo 2004). However, *trans*-resveratrol had a weaker DPPH radical scavenging capacity than quercetin, catechin, epicatechin, and rutin according to their IC_{50} values against DPPH radicals (Iacopini *et al*. 2008).

It needs to be pointed out that grape phenolic antioxidants have shown other beneficial effects besides their antioxidative properties. These beneficial effects may include but are not limited to anti-proliferative activity against cancer cells (Parry *et al*. 2006; Choi and Lee 2008), antibacterial activity (Jayaprakasha *et al*. 2003; Luther *et al*. 2007), lifespan extension (Howitz *et al*. 2003; Wood *et al*. 2004), and prevention of cataract formation (Yamakoshi *et al*. 2002). These beneficial properties may not be mediated by their antioxidant activities, but rather by other metabolic pathways.

EFFECTS OF POST-HARVEST TREATMENTS ON GRAPE SEED ANTIOXIDANTS

Effects of thermal treatment

The bioavailability of bioactive food factors is critical for their beneficial effects. The bioavailability depends on their original concentration in the raw ingredients and changes during post-harvest treatments, such as chemical and biochemical reactions during storage and ingredient processing, as well as their interactions with other components during food formulation and processing. Effects of thermal treatment on antioxidant availability in grape seeds has been investigated (Kim *et al.* 2006). Whole and powdered grape seeds (*V. vinifera*, Campbell early) were heated at 50, 100, 150, or 200 °C for 10, 20, 30, 40, 60, 90, and 120 minutes. These seed preparations were compared to the control, which are the seeds without thermal treatment, for their

Table 5 Effects of thermal treatment on total phenolic content (mM TAE) of whole and powdered grape (*V. vinifera*, Campbell early) seeds.[a]

Temperature (°C)	Heating time (min)							
	0	**10**	**20**	**30**	**40**	**60**	**90**	**120**
WGSE								
50	0.380	0.317	0.260	0.303	0.300	0.313	0.442	0.330
100	0.380	0.326	0.347	0.414	0.407	0.520	0.458	0.390
150	0.380	0.392	0.348	0.444	0.575	0.484	0.358	0.319
200	0.380	0.254	0.189	0.163	0.115	0.179	0.179	0.113
PGSE								
50	0.380	0.344	0.332	0.451	0.424	0.444	0.400	0.515
100	0.380	0.555	0.296	0.359	0.375	0.378	0.418	0.185
150	0.380	0.340	0.417	0.427	0.483	0.407	0.319	0.196
200	0.380	0.190	0.185	0.269	0.160	0.151	0.067	0.064

[a] Referenced from Kim *et al.* 2006

TAE: tannic acid equivalents; WGSE: whole grape seed extract; PGSE: powdered grape seed extract.

TPC and antioxidant properties. As shown in **Table 5**, TPC values decreased in both whole and powdered grape seeds in a temperature and time dependent matter except the powdered seeds kept at 50 °C, indicating the possible loss of phenolic components due to thermally induced chemical reactions, such as oxidation. It should also be noted that powdered grape seeds demonstrated a higher TPC value than the whole seed counterparts at all time points when kept at 50 and at 100 °C for 10 min, but the whole seeds had a greater TPC value than the powdered seed counterparts when they were kept at 150 and 200 °C for 10-120 min, or at 100 °C for 20-120 min. These results suggest that mild thermal treatment, such as heating at 50 °C for a short time period, may enhance the extractable or available level of phenolics in the powdered grape seeds. This also suggests that the seed matrix may protect phenolic compounds during thermal treatment. This observation may be explained by the overall effects of thermal cleavage of associations between phenolics and the seed matrix, and the increased surface area of the powdered seeds (Meyer *et al.* 1997; Cheng *et al*. 2006; Kim *et al.* 2006). Heat treatment has been shown to possibly increase release of phenolic compounds as it may convert insoluble bound phenolic compounds into soluble phenolic compounds (Kim *et al.* 2006; Moore *et al*. 2009).

Thermal treatment also altered the antioxidant property of grape seeds (Kim *et al*. 2006). As shown in **Fig. 4**, increase of the extractable DPPH radical scavenging capacity was observed in whole and powdered grape seeds kept at 50 and 100 °C for 60 and 120 min, while heating at 200 °C for 60 and 120 min decreased DPPH radical scavenging activity in whole and ground seeds (Kim *et al*. 2006). Kim *et al*. (2006) also reported the alteration of reducing power of grape seeds by thermal treatment.

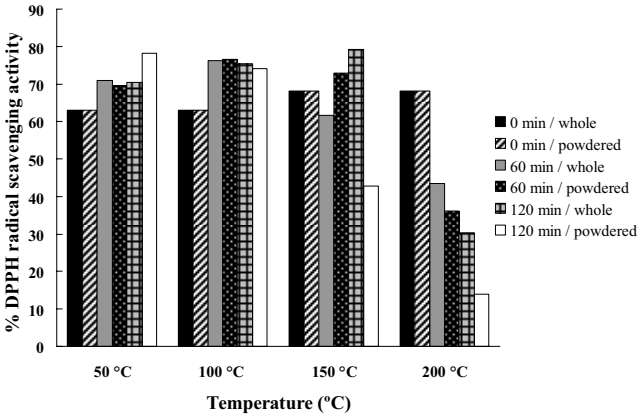


Fig. 4 Effects of thermal treatment and particle size on antioxidant stability in whole and powdered grape seeds (adapted from Kim *et al.* 2006). The radical scavenging activity was estimated by the formula: % DPPH radical scavenging activity = (1-sample absorbance/control absorbance) × 100%.

Effects of particle size

Particle size of the botanical materials including food and nutraceutical ingredients may affect the stability of their important components during storage and post-harvest treatments such as ingredient and food processing procedures (Cheng *et al*. 2006). In addition to the observation by Kim *et al.* (2006) that reduction of particle size, grinding grape seeds, might contributed to the increased available amount of total phenolics kept at 50°C (see *Effect of Thermal Treatment* section), Meyer *et al.* (1997) reported that crushed seeds might have higher level of extractable amount of total phenolics, benzoic acids, flavanols, and cinnamates, but not anthocyanins, than their whole seeds counterparts (Meyer *et al.* 1997). Under the same analytical conditions, crushed Cabernet Sauvignon grape seeds had a TPC value of 1780 mg GAE/L and the TPC was 565 mg GAE/L for the whole seeds (**Table 6**). As shown in **Table 6**, crushing increased both extractable TPC and total flavanols for Cabernet Sauvignon seeds and Petite Sirah late grape seeds. However, crushing had little effect on anthocyanin availability in Cabernet Sauvignon seeds, and reduced the anthocyanin availability in Petite Sirah late grape seeds (**Table 6**). These data indicated that effect of post-harvest treatment on seed antioxidant properties may depend on the grape cultivar. Another study by Bucić-Kojić *et al.* found that out of four particle sizes, 0.125-0.16, 0.16-0.4, 0.4-0.63, and > 0.63 mm, the smallest particle size might result in the highest extractable or available TPC content (Bucić-Kojić *et al.* 2006).

In addition, particle size also altered antioxidant properties of grape seeds. Kim *et al*. (2006) reported that powdered grape seeds had lower DPPH radical scavenging capacity than its counterpart when heated at 200°C for 60 and 120 min (**Fig. 4**). Interestingly, powdered seed had greater DPPH radical scavenging capacity than the whole seeds when heated at 150°C for 60 min, but the whole seeds had stronger radical scavenging ability when heated at 150°C for 120 min.

Effects of storage conditions

Storage conditions have been found to affect antioxidant properties in grapes and grape seeds (Hatzidimitriou *et al.* 2007; Romero *et al.* 2008). Hatzidimitriou *et al.* (2007) analyzed the effects of storage at three different relative humidity (RH) levels, 33, 53, and 75%, on TPC of grape seed extracts. TPC decreased from 438 to 327 and 438 to 344 mg GAE/g dry extraction respectively for the grape seeds kept at 33 and 53% RH in 50 days, and dropped from 438 to 234 mg GAE/g for seeds stored at 75% RH (Hatzidimitriou *et al.* 2007). DPPH$^{\bullet}$ scavenging abilities of the grape seed extracts were slightly reduced at all tested RH conditions. Interestingly, storage at either higher RH level or for longer time could enhance the gallic acid level in the grape seeds,

Table 6 Effects of different extraction times and crushing on total phenolic, anthocyanin, and flavanol contents of grape seed extracts.[a]

Extraction time	Total phenolics (mg GAE/L)	Anthocyanins (mg ME/L)	Flavanols (mg CCE/L)
Cabernet Sauvignon (whole seeds)			
1 min	565	718.0	0.0
1 h	686	696.0	0.0
4 h	771	793.8	0.0
24 h	737	705.8	8.9
165 h (≈ 7 days)	890	746.4	0.0
Cabernet Sauvignon (crushed seeds)			
1 min	1780	791.7	133.7
1 h	1868	879.9	122.7
4 h	1930	867.8	140.9
24 h	2015	856.2	156.0
165 h (≈ 7 days)	2138	775.2	167.4
Pinoir Sirah late (whole seeds)			
1 min	1115	1708.2	5.9
1 h	1136	1483.6	0.0
4 h	1163	1558.9	5.5
24 h	1183	1477.3	21.5
165 h (≈ 7 days)	1367	1564.7	99.4
Pinoir Sirah late (crushed seeds)			
1 min	1741	1337.2	93.4
1 h	1820	1644.2	101.8
4 h	1966	1463.4	137.6
24 h	1964	1513.2	173.6
165 h (≈ 7 days)	2094	1344.0	168.0

[a] Data from Meyer *et al.* 1997

GAE: gallic acid equivalents; ME: malvin equivalents; CCE: catechin equivalents.

but would reduce both catechin and epicatechin contents (Hatzidimitriou *et al.* 2007).

CONSIDERATIONS IN ANTIOXIDANT PROPERTY ESTIMATION FOR GRAPE SEEDS

Many factors may alter the overall estimation of antioxidant properties of grape seeds. It is widely accepted that mistakes made during sample preparation can not be corrected in the later analytical steps. In 2005, Pinelo *et al.* investigated the effects of solvent, temperature, and solvent-solid ratio on estimation of total phenolic content and radical scavenging capacity of grape pomace, stem, seeds, and skin. The extraction temperature and solvent-solid ratio were critical in the extraction efficiency of phenolic antioxidants (Pinelo *et al.* 2005). Also noted was that methanol was most effective for phenolic extraction, while ethanol extracted the highest level of soluble material under the experimental conditions, suggesting the importance of solvent type in antioxidant property estimation. This conclusion was supported by findings from a recent study (Yilmaz and Toledo 2006). Results from this study showed that methanol, ethanol, and acetone with different levels of water differed in their capacities in extracting phenolic components from grape seeds and skin (Yilmaz and Toledo 2006). The critical role of temperature and solvent-solid ratio on phenolic extraction from grape seeds was confirmed by a kinetic study performed by Bucić-Kojić *et al*. (2007). In addition, pH and particle size might alter the extraction efficiency of phenolic antioxidants from grape seeds (Janisch *et al.* 2006; Makris *et al.* 2006; Bucić-Kojić *et al*. 2007).

SUMMARY

Grape seeds and seed fractions may serve as dietary source of natural phenolic antioxidants, such as flavanols, anthocyanins, and simple phenolic acids. Genotype, growing conditions, and post-harvest treatments may alter the availability of antioxidants in grape seeds. This may be a challenge in developing grape seeds-based nutraceutical ingredients for human utilization. Multi-mechanisms such as radical scavenging and chelating reactions may be involved in the antioxidative actions of these phenolic compounds. Additional research is required to investigate their health beneficial effects and possible side effects to promote their application in improving human health.

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