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SCIENCE DIRECT®

Food Chemistry 95 (2006) 1–8

Food Chemistry

www.elsevier.com/locate/foodchem

Evaluation of free radical-scavenging properties of commercial grape phenol extracts by a fast colorimetric method

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Received 16 August 2004; received in revised form 6 December 2004; accepted 6 December 2004

Abstract

Three commercial extracts of grape phenols (seeds, skin, whole) were used in this study. Each extract was divided by HPLC into five fractions of different polarities, and their free radical-scavenging activities were measured using the DPD (*N*,*N*-diethyl-*p*-phenylenediamine) colorimetric method. Total phenol contents were determined using the Folin-Ciocalteu method to assess their contribution to the antiradical activity. The results showed that there was good correlation between total phenolic compound contents and free radical-scavenging activities of the different grape extracts. Moreover, the seed extract presented the most important free radical-scavenging properties (138 USP/mg extract), whereas the whole extract presented the lowest free radical-scavenging capacity (80.5 USP/mg extract). However, the free radical-scavenging properties, reported on the basis of content of phenolic compounds in each extract, showed that the free radical-scavenging activities of seed and whole extracts were not significantly different (171 versus 162 USP/mg phenol). In addition, free radical-scavenging activities of fractions from seed and skin extracts decreased as their polarities decreased.
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Keywords: Grape extracts; Total phenols; HPLC fractions; Free radical-scavenging capacity; DPD method

1. Introduction

Phenolic compounds are naturally-occurring substances in fruits, vegetables, nuts, seeds, flowers and also some herbs, in which they contribute to colour and sensory properties, such as bitterness and astringency (Macheix, Fleuriet, & Billot, 1990). The growing interest in the antioxidant properties of the phenolic compounds in vegetables and fruits derives from their strong activity and low toxicity compared with those of synthetic phenolic antioxidants, such as BHT (butylated hydroxytoluene) (Marinova & Yanishlieva, 1997; Nakatani, 1996). These important compounds are an integral part of the human diet, and could be helpful against human cancers, arteriosclerosis, ischaemia and inflammatory disease, which are partially caused by exposure to oxidative stress (Halliwell, 1996; Namiki, 1990). Indeed, epidemiological studies have shown that consumption of food and beverages rich in phenolics, such as tea and wine, is correlated with reduced coronary heart disease mortality (Balentine, Wiseman, & Bouwens, 1997; Cul, Juhasz, & Tosaki, 2002; Hertog et al., 1995; Serafini, Laranjinha, Almeida, & Mainai, 2000). The protective effects of vegetable, fruit, and beverage consumption against coronary artery disease and certain types of cancer are partly attributed to the flavonoid content of these foods (Bell et al., 2000; Frankel, Kanner, German, Parks, & Kinsella, 1993). It has been demonstrated,

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0308-8146/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.12.011

both in vitro and in vivo that these phenolic compounds can offer significant anti-atherogenic protection by inhibiting the oxidation of low density lipoproteins (LDLs) (Rice-Evans, Miller, Bolwell, & Bramley, 1995; Serafini et al., 2000; Vinson, 1998). Nigdikar, Williams, Griffin, and Howard (1998) study provided additional support for protective effects of phenolic antioxidants on cardiovascular disease (CVD).

The presence of bioactive compounds in grapes, mainly phenolic compounds, and the synergistic effects among them, have been related to these properties (Frankel, Waterhouse, & Teissedre, 1995; Soleas, Diamendis, & Goldberg, 1997). The phenolic compounds in grapes range from simple compounds (monomers) to complex tannin-type substances (oligomers and polymers). The antioxidant compounds present in grape have been identified as phenolic acids (benzoic and hydroxynnamic acids), stilbene derivatives (resveratrol), flavan-3-ols (catechin, epicatechin), flavonols (kaempferol, quercetin, myricetin) and anthocyanins (Ghiselli, Nardini, Baldi, & Scaccini, 1998; Miller & Rice-Evans, 1995; Vinson & Hontz, 1995). One of the most abundant of these phenolic compounds is the flavan 3-ol compound, catechin (Singleton, 1988; Singleton & Essau, 1969). The flavan 3-ols are mainly localised in the seeds and the skin (Thorngate and Singleton, 1994), although traces of monomers and dimers have been detected in the pulp (Bourzeix, Weyland, Hereidia, & Desfeux, 1986; Ricardo Da Silva, Rosec, Bourzeix, Mourgues, & Moutonet, 1992). The phenolic acids of grape are hydroxycinnamic acids which are in the form of esters of the tartaric acid in the skin and pulp (Ribereau-Gayon, 1965). On the other hand, the flavonols present in the white and red grape are localised only in the skin (Cheynier & Rigaud, 1986; Wulf & Nagel, 1980). Similarly, the stilbene derivatives are only located in the skin of the grapes (Jeandet, Bessis, & Gautheron, 1991; Lamuela-Raventos, Romero-Perez, Waterhouse, & de la Torre-Boronat, 1995). The anthocyanidins, present only in red grapes, are generally localised in the skin (Amrani-Joutei, 1993) and, for some type of vines, in pulp (Pecket & Small, 1980). The procyanidin composition of grape seeds has been determined (Lee & Jaworski, 1997). Escribano-Bailon, Gutierrez-Fernandez, Rivas-Gonzalo, and Santos-Buelga (1992) have reported 17 chemical constituents in *Vitis vinifera* grape seeds. Gabetta et al. (2000) reported the presence of monomers to heptamers and their gallates in grape seeds.

Antioxidants can interfere with the oxidation process by reacting with free radicals, chelating catalytic metals, and also by acting as oxygen scavangers (Shahidi & Wanasundara, 1992). Four commonly used methods to evaluate the antioxidant activity in food are the ferric thiocyanate (FTC), the thiobarbituric acid reactive substances (TBARS), the oxygen radical absorbance capacity (ORAC), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) procedures. The FTC and TBARS methods measure the antioxidant activity by the inhibition of lipid oxidation. The DPPH and ORAC methods measure the free radical-scavenging capacity of the antioxidant. Although the antioxidants of grape and wine in lipid systems have been extensively studied (Frankel et al., 1993, 1995; Fuhrman, Volkova, Suraski, & Aviram, 2001; Kanner, Frankel, Granit, German, & Kinsella, 1994; Sanchez-Moreno, Larrauri, & Saura-Calixto, 1999; Teissedre, Frankel, Waterhouse, Peleg, & German, 1996), investigations on the free radical-scavenging capacity of grapes are scarce (Ahn et al., 2002; Ghiselli et al., 1998; Jayaprakasha, Selvi, & Sakariah, 2003).

Therefore, the objective of this work was to determine the free radical-scavenging capacity of three commercial phenolic extracts from grape (whole, seed, skin) and those fractions of different polarity obtained from the three extracts. In this work, an HPLC method for the separation of fractions was established and a rapid colorimetric method for measurement of free radical-scavenging capacity was applied.

2. Materials and methods

2.1. Raw material

Three grape extracts provided by Polyphenolics (Madera, CA, USA) were used in this study. These extracts were MegaNatural™ Whole Grape Extract, MegaNatural™ Gold Grape Seed Extract and GSKE grape skin extract. The extracts were defined as dry powder and, according to provider, total phenolics concentration ranged from 45 to 90 g of gallic acid equivalents (GAE) per 100 g of extract. The MegaNatural™ Whole Grape Extract, which comes from the Rubired grape, had an anthocyanin content of 25% (w/w) and total phenol content of 45–50%. The MegaNatural™ Gold Grape Seed Extract, which comes from French Colombard, Chenin Blanc and some Chardonnay grapes, guarantees a minimum 80% (w/w) of phenolic compounds and a standardized composition of monomers, oligomers and polymers. The GSKE grape skin extract deriving from the Zinfandel grape contains approximately 80% (w/w) of phenols and 1.5% of anthocyanins.

2.2. HPLC-DAD fractionation of extracts

The HPLC analyses were performed on a ProStar 230 (Varian Canada Inc., Mississauga, ON, Canada), equipped with a ternary pump delivery system, a Rheodyne injection valve (500 μl capacity, Waters Ltd., Dorval, QC, Canada) and a ProStar 330 diode-array UV–Vis detector (Varian); integration and data

elaboration were performed using Star Chromatography Workstation software (Varian). A Prep Nova-Pak® HR C_{18}, 6 μm, 7.8 × 300 mm (Waters) column was used. All solvents were filtered with a 0.45 μm Millipore (Millipore Canada Ltd., Etobicoke, ON, Canada) filter disk and degassed with helium. A gradient elution was carried out using the following solvent systems: mobile phase A, double-distilled water/acetonitrile/formic acid (94.9/5/0.1, v/v/v); mobile phase B, double-distilled water/acetonitrile/formic acid (69.9/30/0.1, v/v/v). The linear gradient elution system was: 100–0% A from 0 to 60 min, keeping 100% B for 10 min, returning to 100% A, followed by equilibration for 10 min before injection. The grape extracts were dissolved in a double-distilled water/acetone/formic acid solution (89.9/10/0.1 v/v/v). For each sample, 500 μl were injected after filtration through a 0.45 μm filter disk. The flow rate was 3 ml min^{-1} and the detection was achieved by photodiode array (250–550 nm) (Fig. 1). Five fractions per extract were recovered (Table 1). The solvent was then removed under nitrogen and dry matter was determined by freeze-drying the fractions for 48 h.

2.3. Total phenol concentration

Total phenolic compound content in each grape extract or fraction was spectrophotometrically determined according to the Folin-Ciocalteu procedure (Singleton & Rossi, 1965) by reading the absorbances at 760 nm, and results were expressed as grams of gallic acid equivalents (GAE) per 100 g of extract.

2.4. Free radical-scavenging capacity

Antioxidative capacity of extracts and fractions were evaluated following a modified procedure of the DPD (*N*,*N*-diethyl-*p*-phenylenediamine) (Sigma-Aldrich Ltd, Oakville, ON, Canada) colorimetric method (APHA, 1989), as reported by others (Le Tien, Vachon, Mateescu, & Lacroix, 2001). Two hundred microlitres of sample were added to a cell containing 3 ml of 0.15 M NaCl and submitted to electrolysis for 1 min (10 mA DC, 400 V) using a power supply (Bio-Rad, model 1000/500, Mississauga, ON, Canada). After electrolysis, an aliquot of 200 μl was added to 2 ml of DPD solution

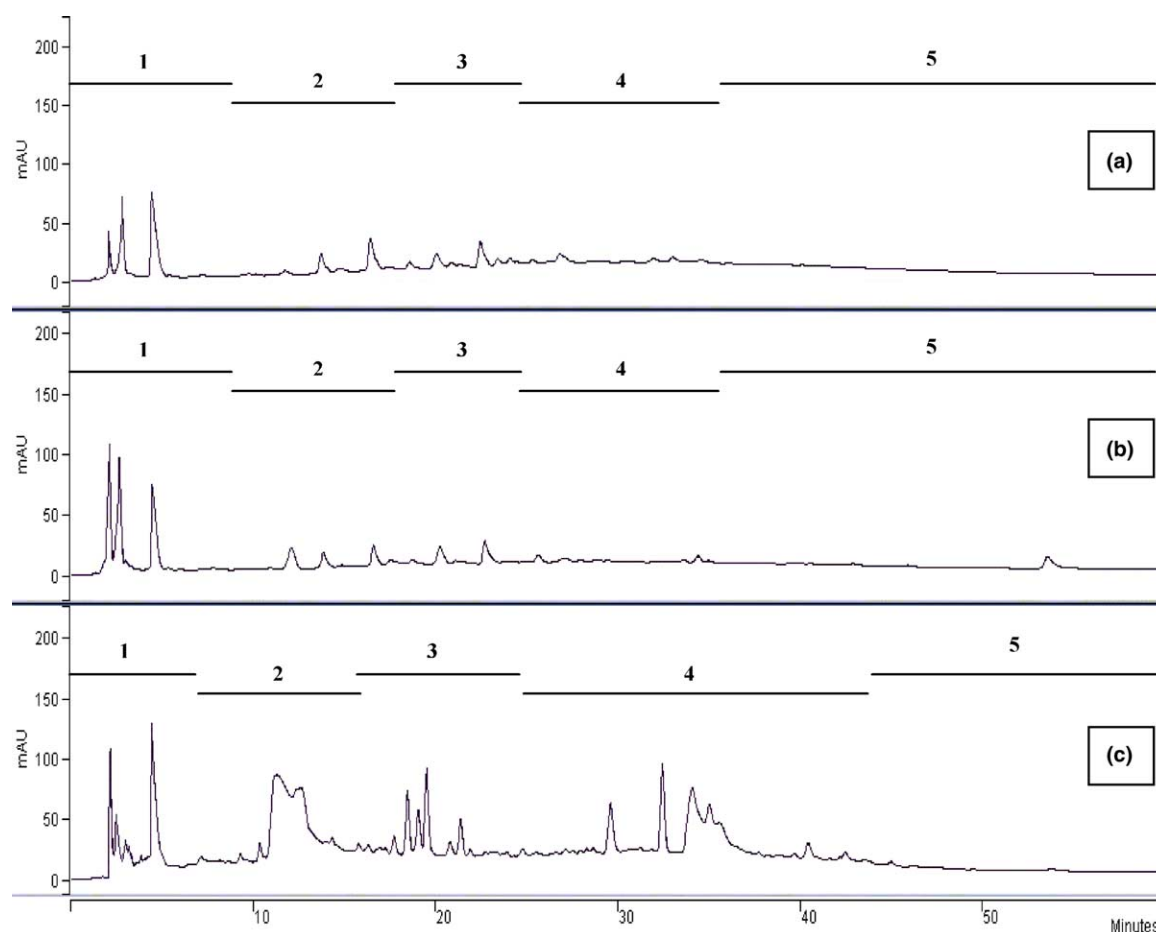

Fig. 1. Chromatogram obtained from three grape extracts (seed (a), skin (b), whole (c)) on a Prep Nova-Pak® HR C_{18} column (300 × 7.8 mm I.D.). Mobile phase was water/acetonitrile/formic acid and a linear gradient was carried out between solvent A (94.9/5/0.1, v/v/v) and the solvent B (69.9/30/0.1, v/v/v). The DAD was set at 250–550 nm. Five fractions (#1–5) of different polarities were separated from each extract.

Table 1
Times of elution and ACN/water percentages used for HPLC-DAD fractionation[a] of commercial grape extracts

Fractions	Whole grape		Grape seeds		Grape skin	
	Time (min)	ACN/water (%)[b]	Time (min)	ACN/water (%)	Time (min)	ACN/water (%)
1	0–7	5.00/94.90–7.91/91.99	0–9	5.00/94.90–8.75/91.15	0–9	5.00/94.90–8.75/91.15
2	7–16	7.91/91.99–11.66/88.24	9–18	8.75/91.15–12.50/87.40	9–	18 8.75/91.15–12.50/87.40
3	16–25	11.66/88.24–15.41/84.49	18–25	12.50/87.40–15.41/84.49	18–25	12.50/87.40–15.41/84.49
4	25–44	15.41/84.49–23.33/76.57	25–36	15.41/84.49–20.00/79.90	25–36	15.41/84.49–20.00/79.90
5	44–60	23.33/76.57–30.00/69.90	36–60	20.00/79.90–30.00/69.90	36–60	20.00/79.90–30.00/69.90

[a] The fractions were defined to obtain well delimited peaks (Fig. 1). The differences (whole grape versus grape seeds or grape skin, respectively) in fractionation were due to the high number of peaks in the whole grape extracts.
[b] Mobile phase was water/acetonitrile/formic acid and a linear gradient was carried out between solvent A (94.9/5/0.1, v/v/v) and the solvent B (69.9/30/0.1, v/v/v) for 60 min.

(25 mg/ml). The generated oxidative species (superoxide anion (O_2^-), singlet oxygen ($^1O_2$) and OH radicals) and their by-products (hydrogen peroxide (H_2O_2) and hypochlorite ion (OCl^-)) react instantly with DPD, producing a red coloration that can be measured at 515 nm using a DMS 100S spectrophotometer (Varian Canada Inc., Mississauga, ON, Canada). The colorimetric reaction was calibrated with FCC/USP ascorbic acid (Laboratoires Denis Giroux Inc., Saint-Hyacinthe, QC, Canada). The antioxidant activity is equivalent to the capacity of grape polyphenols to inhibit the accumulation of oxidative species (able to oxidize DPD) and, consequently, the red coloration at 515 nm. The reaction advancement was quantified using the non-electrolyzed NaCl solution (no oxidative species, ascribed to 100% scavenging) and the electrolyzed NaCl solution (0% scavenging, in the absence of any antioxidants). The scavenging percentage was calculated according to the following equation:

$$\text{Scavenging}\ (\%) = 100 - [(\text{OD}_{\text{sample}}/\text{OD}_{\text{control}}) \times 100]$$

where $\text{OD}_{\text{control}}$ represents the OD of electrolyzed solution in the absence of sample. In fact, OD is directly related to the degree of oxidation of DPD reagent by the oxidative species. Thus, extracts or fractions able to completely reduce the level of reactive oxidative species will have a 100% scavenging capacity.

The antiradical activity of extracts and fractions was estimated from a calibration curve ($r^2 = 0.9886$) by plotting known solutions of USP ascorbic acid (0.6, 1.2, 1.8, 2.4 and 3 units USP) against % scavenging capacity. One USP (United States Pharmacopeia) unit is the free radical scavenging activity of 0.05 mg of the USP ascorbic acid reference standard. Then, data were related to the quantity of phenolic compounds and results were expressed as USP/mg of phenol. The mean antiradical activity RSD for reproducibility was 6.4%.

2.5. Statistical analysis

Analysis of variance and Duncan's multiple-range tests were employed to statistically analyze all results. Differences between means were considered significant when $p \leqslant 0.05$. Stat-Packets Statistical Analysis software (SPSS Base 10.0, SPSS Inc. Chigaco, IL, USA) was used for the analysis. For each measurement, three samples in each replicate were tested.

3. Results and discussion

The free radical-scavenging capacity and total phenolic compound content in each grape extract are presented in Table 2. The results showed that the phenolic concentrations in the seed and skin grape extracts were 80.7 and 79.2 g GAE/100 g, respectively. These concentrations were 1.6 times higher than that of the whole extract with a value of 49.6 g GAE/100 g.

Table 2
Radical-scavenging capacity and total phenolic compound content in each grape extract

Grape extracts	Total phenols[a]	Free radical scavenging capacity[a]	
	(g GAE/100 g)[b]	(USP/mg extract)[c]	(USP/mg phenol)[c]
Seed	80.7 ± 1.70b	138 ± 11.2c	171 ± 13.8b
Skin	79.2 ± 1.12b	100 ± 5.69b	126 ± 7.18a
Whole	49.6 ± 1.19a	80.5 ± 4.05a	162 ± 8.16b

[a] Means in the same column bearing the same letter are not significantly different ($p > 0.05$).
[b] Gallic acid equivalents, dry basis.
[c] USP (United States Pharmacopeia) unit.

These results are in agreement with the phenolic concentrations given by the supplier of the grape extracts. The seed extract presented the most significant free radical-scavenging activity (138 USP/mg extract), whereas the whole extract presented the lowest free radical-scavenging capacity (80.52 USP/mg extract). However, the free radical-scavenging activity, reported on the basis of phenolic compounds content in each extract, showed that the free radical-scavenging capacities of seed and whole extracts were not significantly different ($p > 0.05$) with respective values of 171 and 162 USP/mg phenol. The skin extract presented the lowest capacity (126 USP/mg phenol) among the three extracts.

Use of electrolysis as a method to generate oxidative stress was first introduced by others (Jackson, Mickelson, Stinger, Rao, & Lucchesi, 1986) for physiological studies on perfused isolated organs. Oxidative damage was realized by electrolysis of Krebs–Henseleit buffer. Le Tien et al. (2001), who sought to measure the free radical-scavenging capacity of edible films, showed that similar oxidative effect can be obtained by electrolysis of 0.15 M NaCl, in the same conditions (10 mA, 400 V for 1 min). In our study, this colorimetric method (DPD) was adapted to measure the free radical scavenging capacity of grape phenols. The degree of discoloration indicated the scavenging potentials of the antioxidant extracts. The activity of the extracts is attributed to their hydrogen-donating ability (Shimada, Fujikawa, & Nakamura, 1992). Antioxidants are believed to intercept the free radical chain of oxidation and to donate hydrogen from the phenolic hydroxyl groups, thereby forming stable end-products, which do not initiate or propagate further oxidation (Sherwin, 1978).

A number of methods for measuring antiradical activity have been reported over recent years. Among them, the FRAP (ferric reducing antioxidant power) (Benzie & Strain, 1996) and the TRAP (total radical-trapping antioxidant parameter) (Wayner, Burton, Ingold, & Locke, 1985) have gained popularity because they are simple and speedy. However, compared to other antiradical methods, these methods have some kinetic or mechanistic flaws (Ou, Hampsch-Woodill, & Prior, 2001). From a methodological point view, the DPPH (2,2-diphenyl-1-picrylhydrazyl) (Gil, Tomas-Barberan, Hess-Pierce, Holcroft, & Kader, 2000) and ORAC (oxygen radical absorbance) (Cao, Alessio, & Culter, 1993) assays are recommended as easy and accurate methods for measuring the antiradical activity of fruit and vegetable juice or extracts. However, the DPPH method is less sensitive than other methods for hydrophilic antioxidants (Gil et al., 2000), while the DPD method can be used as well with hydrophilic and hydrophobic antioxidants in water as in alcohol (Oussalah, Caillet, Salmiéri, Saucier, & Lacroix, 2004). Also, the ORAC assay cannot be considered a ≪total antiradical activity assay≫, since it primarily measures antiradical hydrophilic activities against peroxyl radicals (Ou et al., 2001). Although DMPD assay (*N*,*N*-dimethyl-*p*-phenylenediamine) is used to measure the antioxidant capacity of wines (Fogliano, Verde, Randazzo, & Ritieni, 1999), this method is not appropriate for lipophilic compounds and requires the use of ABTS (2,2′-azinobis(3-ethylbenzthiazoline)-6-sulfonic acid) for lipophilic constituents (Sanchez-Moreno, 2002; Scalfi et al., 2000). The ABTS (Antolovich, Prenzler, Patsalides, McDonald, & Robards, 2002) and DPPH methods are substrate-free. Their popularity can be attributed to simplicity and speed of analysis, but this is achieved at a potential price and the relevance of data generated with these procedures must be considered carefully (Antolovich et al., 2002). The results obtained with the DPD method were highly reproducible; the precision, which is expressed as mean relative standard deviation (%RSD) for all samples, was 6.4%. Also, the DPD method is very simple, inexpensive and, especially, fastest of all the methods for measuring the antiradical activity, since the sample was submitted to electrolysis for 1 min. The reaction times are 4 min for the FRAP assay, 10 min for the ABTS method and DMPD assay, and 15 min for the DPPH method. The ORAC assay is even longer and more complex because of the fluorescence reading.

The data obtained reveal that the grape extracts are free radical-scavengers and primary antioxidants, which react with free radicals. However, these results indicate that the phenols of the seed extract have free radical-scavenging activities which are more significant than those of phenols present in the skin extract, and this in spite of the fact that the red grape skin contains phenolic substances which embrace many classes of compounds, ranging from phenolic acids, coloured anthocyanins and simple flavonoids to complex flavonoids (Amrani-Joutei, 1993; Cheynier & Rigaud, 1986; Lamuela-Raventos et al., 1995; Ribereau-Gayon, 1965); Ricardo Da Silva et al., 1992; (Thorngate and Singleton, 1994). On the other hand, Escribano-Bailon et al. (1992) have reported 17 chemical constituents in *Vitis vinifera* grape seeds which all are the monomers or polymers of flavan-3-ols. The major compounds are (+)-catechin (11%), (−)-epicatechin (10%), (−)-epicatechin-3-*O*-gallate (9%), epicatechin 3-*O*-gallate- ($4\beta \rightarrow 8$)-catechin (dimer B1-3-*O*-gallate) (7%) and epicatechin-($4\beta \rightarrow 8$)-epicatechin (dimer B2) (6%). Fuleki and Ricardo da Silva (1997) have reported monomers of (+)-catechin, (−)-epicatechin, and (−)-epicatechin-3-*O*-gallate, 14 dimeric, 11 trimeric procyanidins and one tetrameric procyanidin from grape seeds. Antiradical activity was reported to be dependent on the structure of the free radical-scavenging compounds, the substituents present on the ring of the flavonoids, and the degree of polymerization. Although there is some debate as to whether the degree of polymerization increases the antiradical capacity, it

appears that epicatechin, epicatechin polymers and the B procyanidins are better antioxidants than catechin and catechin polymers (Ricardo da Silva, Darmon, Fernandez, & Mitjavila, 1991; Saint-Crizq de Gaulejac, Provost, & Vivas, 1999). The structural criteria for the potent free radical-scavengers are that these should possess either (i) a 3-hydroxy group on an unsaturated C ring or (ii) a 2,3-double bond with the 3-OH group and 4-one in the C ring or (iii) an ortho-OH substitution pattern in the B ring where the OH groups are not glycosylated (Rice-Evans et al., 1995; Rice-Evans, Miller, & Paganga, 1996). The major polyphenolic components in grape seeds are catechin, epicatechin, and procyanidins (Jayaprakasha, Singh, & Sakariah, 2001), which fulfill the first and third structural criteria for being good antioxidants.

HPLC analysis (Fig. 1) allowed separation of the phenolic compounds of three grape extracts according to their polarity. The most polar phenolic compounds presented the shortest elution times. Five fractions from each grape extract were collected according to conditions defined in Table 1, in order to collect compounds with close polarities. The free radical-scavenging activity of these fractions is presented in Table 3. The most polar fractions of seeds or skin showed the highest free radical-scavenging activities. Among the fractions from seeds, fraction 1 presented the highest free radical-scavenging capacity (101 USP/mg phenol), followed by fraction 2 (68.6 USP/mg phenol). Fractions 1 (62.7 USP/mg phenol) and 2 (69.9 USP/mg phenol) of the skin extract showed free radical-scavenging activities which were at least twice higher than those of the other fractions of the extract. On the other hand, except for fraction 2, all the fractions of the whole extract showed an important free radical-scavenging activity. Moreover, fraction 3 had the highest free radical-scavenging capacity (220 USP/mg phenol).

Table 3
Antiradical capacity of each fraction from three grape extracts

Grape extracts	Fractions	Free radical-scavenging capacity (USP/mg phenol)[a,b]
Seed	1	101 ± 10.6g
	2	68.6 ± 8.56f
	3	40.5 ± 0.46c
	4	53.9 ± 1.36de
	5	42.2 ± 4.00c
Skin	1	62.7 ± 3.71ef
	2	69.9 ± 6.97f
	3	21.2 ± 0.22a
	4	23.6 ± 0.22ab
	5	34.3 ± 0.33bc
Whole	1	90.8 ± 7.18g
	2	43.6 ± 5.76cd
	3	220. ± 21.1i
	4	122 ± 8.28h
	5	99.7 ± 4.01g

[a] Means bearing the same letter are not significantly different ($p > 0.05$).
[b] USP (United States Pharmacopeia) unit.

According to these results, it appears that the polarity of phenolic compounds is a determinant of free radical-scavenging capacity. It is known that the polyhydroxylated phenolic compounds have a higher polarity than those of the other phenols (Heim, Tagliaferro, & Bobilya, 2002; Vasserot, Caillet, & Maujean, 1997). The polarity of the flavonoids depends primarily on the nature of the radicals on rings, and in particular on the number of OH groups. The glycosylated derivatives also have an influence on the polarity of the molecule. Thus, the spatial arrangement of substituents is a greater determinant of antiradical activity than the flavan backbone alone (Heim et al., 2002; Rice-Evans et al., 1996). Consistent with most polyphenolic antioxidants, both the configuration and total number of hydroxyl groups substantially influence several mechanisms of antiradical activity (Burda & Oleszek, 2001; Cao, Sofic, & Prior, 1997; Sekher Pannala, Chan, O'Brien, & Rice-Evans, 2001). Free radical-scavenging capacity is primarily attributed to the high reactivities of hydroxyl substituents. The B-ring hydroxyl configuration is the most significant determinant of scavenging of reactive oxygen species (Burda & Oleszek, 2001; Sekher Pannala et al., 2001). Hydroxyl groups on the B-ring donate hydrogen and an electron to hydroxyl, peroxyl, and peroxynitrite radicals, stabilizing them and giving rise to a relatively stable flavonoid radical. Among structurally homologous flavones and flavanones, peroxyl- and hydroxyl-scavenging increases linearly and curvilinearly, respectively, according to the total number of OH groups (Cao et al., 1997). The differences in antiradical activity between polyhydroxylated and polymethoxylated flavonoids are most likely due to differences in both hydrophobicity and molecular planarity (Heim et al., 2002).

4. Conclusion

The free radical-scavenging capacity of grape extracts and phenolic fractions extracted from grape extracts was easily measured using a fast colorimetric method. This assay is cheaper and less laborious than other methods involving the addition of an antioxidant to a radical-generating system (Antolovich et al., 2002; Sanchez-Moreno, 2002). The results obtained by the DPD method were highly reproducible (RSD: 6.4%) and showed that the latter is sensitive to grape phenolic compounds of different polarities. The seed extract had the highest concentration of phenolic compounds among the three extracts, and presented the most significant free radical-scavenging activity. However, the free radical-scav-

enging activity, reported on the basis of phenolic compounds content in each extract, showed that the free radical-scavenging capacities of seed and whole extracts were not significantly different ($p > 0.05$). Even though extracts and fractions showed appreciable free radical-scavenging activities, the most effective fraction (220 USP/mg phenol) was 10-times more active than the least effective fraction (21.2 USP/mg phenol). Also, the most effective fraction, with 220 USP/mg phenol, was 35% more active than the corresponding whole extract, with 162 USP/mg phenol. Our results thus suggest that even a partial purification of the grape phenolic compounds has an important impact on free radical scavenging activities of these compounds.

Acknowledgement

The authors are grateful to Julie Auclair for her technical collaboration.

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