Catechin and Procyanidin Composition of Seeds from Grape Cultivars Grown in Ontario

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The quantities of 11 flavan-3-ols were determined using HPLC in the seeds of 17 vinifera, hybrid, and labrusca type red and white grape cultivars grown in the Niagara region of Ontario, Canada. The ranges of concentrations (mg/100 g of seeds) were as follows: (+)-catechin, 21−244; (−)-epicatechin, 23−284; B1, 3−62; B2, 9−106; B3, trace−71; B4, 2−149; B1-3-O-gallate, trace−74; B2-3-O-gallate, trace−26; B2-3′-O-gallate, trace−11; C1, 0−10; T2, trace−76. The monomers (+)-catechin and (−)-epicatechin were present in most cultivars in greater quantities than any one of the procyanidins. In all but three cultivars, B2 was the most abundant procyanidin and the galloylated procyanidins were present in considerably lower concentrations than the nongalloylated ones. Cultivar influenced the flavan-3-ol composition of the seeds. Gamay, Pinot noir, Baco noir, and Vincent seed were the best sources for these compounds among the cultivars examined.

Keywords: *Nutraceuticals; flavan-3-ols; viniferas; hybrids; labruscas*

INTRODUCTION

Grape seeds are a rich source of catechins and procyanidins (PC). In addition to the monomers (+)-catechin (Cat), (−)-epicatechin (Ec), and (−)-epicatechin 3-O-gallate, 14 dimeric, 11 trimeric, and one tetrameric PC have been identified in grape seeds. Nine of these PC are esterified with one or two gallic acid molecules attached to an Ec unit (Table 1). Substantial quantities of highly polymerized PC are also present. Prieur et al. (1994) found that 55% of the PC in grape seeds consisted of more than five monomer units. Only PC type proanthocyanidins were detected in the seeds while the skin of the grapes contained prodelphinidin as well as PC (Escribano-Bailón et al., 1995). In addition to the PC, four unidentified hydrolyzable tannins were also found in grape seeds (Santos-Buelga et al., 1995).

Grape seeds contribute to the Cat, Ec, and PC contents of red wine and grape juice. These compounds affect the color, clarity, taste, mouth feel (astringency), stability, and aging characteristics of these beverages. Furthermore, catechins and PC are nutraceuticals, contributing to the healthful properties of red wine and grape juice, which are excellent dietary sources of these compounds. Catechins are absorbed into the bloodstream (Das, 1971; Hackett et al., 1983; Midorikawa et al., 1983), exhibit antioxidant (Mangiapane et al., 1992; Scott et al., 1993; Vinson et al., 1995; Teissedre et al., 1996) and free radical scavenging activity (Ricardo da Silva et al., 1991b; Arpentine et al., 1992; Frankel et al., 1993; Hu et al., 1995), and act as antihepatoxic (Perrissoud, 1986; Lacaille-Dubois and Wagner, 1992), antiviral (Selway, 1986), and anticarcinogenic (Liu and Castonguay, 1991) agents.

Masquelier (1982, 1988) suggested that PC stabilize the collagen in the arterial wall, inhibit localized histamine formation in the arteries, and accelerate the removal of cholesterol. Dimeric PC are absorbed into the bloodstream (Das, 1971; Laparra et al., 1977; Masquelier, 1982, 1988; Jimenez-Ramsey et al., 1994), while the higher oligomers pass through the digestive system unabsorbed (Jimenez-Ramsey et al., 1994). Procyanidins act as antioxidants (Ozaki et al., 1990; Mangiapane et al., 1992; Teissedre et al, 1996), scavenge free radicals (Uchida et al., 1987; Ariga and Hamano, 1990; Elstner and Kleber, 1990; Ozaki et al., 1990; Ricardo da Silva et al., 1991b; Arpentine et al., 1992; Frankel et al., 1993; Maffei Facino et al., 1994; Tanahashi et al., 1995), spare vitamin C (Laparra et al., 1979; Masquelier, 1982, 1988), act as antihypertensive (Meunier et al., 1987; Terencio et al., 1991), anti-inflammatory (Gábor, 1986), antimutagenic (Liviero et al., 1994), and antiviral (Takechi et al., 1985) agents, and inhibit some undesirable enzymatic activities (Maffei Facino et al., 1994). Laparra et al. (1979) recommended that 100 mg of PC should be consumed every day. Recognition of the health benefits of catechins and PC initiated the manufacture of grape seed extracts (e.g., Pycnogenols) as dietary supplements.

There are a number of reports available on the Cat, Ec, and individual PC content of mature grape seeds from France (Bourzeix et al., 1986a,b; Romeyer et al., 1986; Dumon et al., 1991; Ricardo da Silva et al., 1991a, 1992b), Yugoslavia (Kovac et al., 1990), Spain (Escribano-Bailón et al., 1992a; Santos-Buelga et al., 1995), Portugal (Ricardo da Silva et al., 1992a), and Morocco (Hmamouchi et al., 1994), but such data is not available from North America. However, only one of these publications provides information on the Cat and Ec (Bourzeix et al., 1986b) and five provide information on the individual PC contents (Bourzeix et al., 1986b; Ricardo da Silva et al., 1991a, 1992a,b; Hmamouchi et al., 1994) expressed as weight of flavanol per unit weight of fresh seed. Furthermore, data are lacking on the flavan-3-ol content of the seeds of interspecific hybrids and labrusca type cultivars which are important in the Eastern grape growing regions of North America. Since

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Table 1. Flavan-3-ols Identified in Grape Seeds

abbreviation	compound	ref[a]
	Monomers	
Cat	(+)-catechin	7
Ec	(−)-epicatechin	7
EcG	(−)-epicatechin 3−O-gallate	7
	Dimer Procyanidins	
B1	Ec-(4β→8)-Cat	3
B2	Ec-(4β→8)-Ec	3
B3	Cat-(4α→8)-Cat	3
B4	Cat-(4α→8)-Ec	3
B5	Ec-(4β→6)-Ec	4
B6	Cat-(4α→6)-Cat	4
B7	Ec-(4β→6)-Cat	1
B8	Cat-(4α→6)-Ec	4
	Dimers Esterified with Gallic Acid	
B1-3-O-gallate	Ec-3-O-gallate-(4β→8)-Cat	4
B2-3-O-gallate	Ec-3-O-gallate-(4β→8)-Ec	4
B2-3′-O-gallate	Ec-(4β→8)-Ec-3-O-gallate	1
B4-3′-O-gallate	Cat-(4α→8)-Ec-3-O-gallate	4
B7-3-O-gallate	Ec-3-O-gallate-(4β→6)-Cat	6
B2-3,3′-di-O-gallate	Ec-3-O-gallate-(4β→8)-Ec-3-O-gallate	4
	Trimer Procyanidins	
C1	Ec-(4β→8)-Ec-(4β→8)-Ec	1
C2	Cat-(4α→8)-Cat-(4α→8)-Cat	5
	Cat-(4α→8)-Cat-(4α→8)-Ec	6
T2	Ec-(4β→8)-Ec-(4β→8)-Cat	1
T3	Ec-(4β→8)-Ec-(4β→6)-Cat	4
T4	Ec-(4β→6)-Ec-(4β→8)-Ec	4
T5	Ec-(4β→8)-Ec-(4β→6)-Ec	4
T6	Ec-(4β→6)-Ec-(4β→8)-Cat	4
	Trimers Esterified with Gallic Acid	
C1-3-O-gallate	Ec-(4β→8)-Ec-(4β→8)-Ec-3-O-gallate	2
T2-3-O-gallate	Ec-(4β→8)-Ec-3-O-gallate-(4β→8)-Cat	4
C1-3,3-digallate	Ec-(4β→8)-Ec-3-O-gallate-(4β→8)-Ec-3-O-gallate	2
	Tetramer Procyanidins	
	Ec-(4β→8)-Ec-(4β→8)-Ec-(4β→8)-Ec	6

[a] Reference given for the first thorough identification of the compound in grape seed: (1) Boukharta (1988); (2) Escribano-Bailón et al. (1992b); (3) Lea et al. (1979); (4) Ricardo da Silva et al. (1991c); (5) Romeyer et al. (1986); (6) Santos-Buelga et al. (1995); (7) Su and Singleton (1969).

the catechins and PC of seeds are important in wine and juice production as well as in utilization of pomace, this study was undertaken to generate data on the Cat, Ec, and individual PC contents of seeds from grape cultivars grown in the Niagara region of Ontario, the major viticultural production area of Canada.

MATERIALS AND METHODS

Hand-harvested mature clusters of 17 grape cultivars/seedlings (cultivars in the following) were obtained from the experimental vineyard of the Horticultural Research Institute of Ontario at Vineland or from the vineyards of the Wiley Brothers Ltd., St. Catharines, Ontario (Foch, Niagara, Vincent) in the 1993 and 1994 growing seasons. The seeds were removed manually from the berries of randomly selected clusters to collect 50−100 g of seeds from each cultivar. The seeds were washed with distilled water, air-dried, and stored at −30 °C until analyzed. The frozen seeds were pulverized in a Waring blender.

Chemical Standards. The chemicals used were of the highest purity commercially available. The source for the Cat and Ec standards was Sarsyntex (Merignac, France). Procyanidins B1, B2, B3, B4, B1-3-O-gallate, B2-3-O-gallate, B2-3′-O-gallate, C1, and T2 were isolated from the methanol extract of grape seeds as described by Ricardo da Silva et al. (1991c). The monomers were quantified as Cat (4.394×10^4 integrator units/μg), dimers and trimers as B2 (2.942×10^4 integrator units/μg), and gallates as B2-3′-O-gallate (5.884×10^4 integrator units/μg).

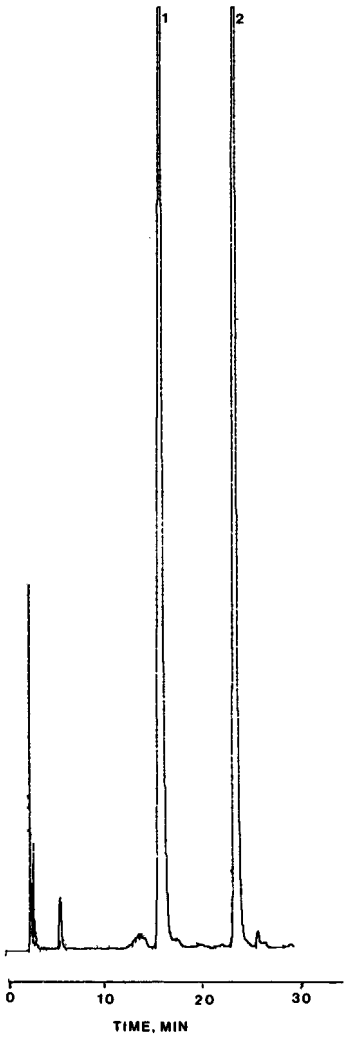

Figure 1. Chromatogram of the catechin fraction [acetonitrile/water (30:70) eluate from the polyamide column] of Riesling grape seed extract monitored at 280 nm. Peak identification (t_R, min): 1, (+)-catechin (15.9); 2, (−)-epicatechin (23.4).

Analytical Procedures. Methanol extracts of the grape seed samples were prepared following the procedure of Bourzeix and co-workers (1986b). The approximately 12 g pulverized seed sample was placed in 50 mL of methanol at −24 °C, protected from light, and extracted under CO_2 for 16 h. The extraction was carried out in a stepwise manner with 50 mL aliquots of solvents; 80% methanol was followed by 50% methanol each for 4 h. Bidistilled water was added to the sample and placed at −24 °C for 15 h. Finally the residue was extracted with 75% acetone for 1 h. Five milliliters of the pooled extracts was purified and fractionated on a polyamide (TLC6, Macherey-Nagel, Düren, FRG) column as described by Ricardo da Silva et al. (1990). The catechins and PC fractions were passed through 0.45 μm membrane filters, and 50 μL aliquots were injected onto the HPLC column.

A Merck Model L-200A pump (Merck-Hitachi, Darmstadt, FRG) was connected to a Waters 717 plus autoinjector (Milford, MA). Detection was made at 280 nm with a Konic UV−vis detector (UVIS 200) coupled to Konichrom data treatment system (Konik Instruments, Miami, FL). The column (250 × 4.6 mm, particle size 5 μm) was a reverse-phase C18 Superspher 100 (Merck, Darmstadt, FRG) protected by a guard column of the same material.

A modification of the method described earlier by Ricardo da Silva et al. (1990) was used for the HPLC separation of

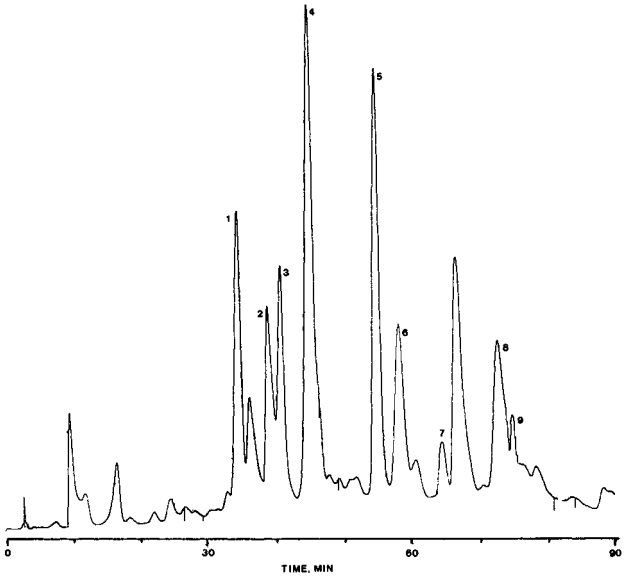

Figure 2. Chromatogram of the procyanidin fraction [acetone/water (75:25) eluate from the polyamide column] of Riesling grape seed extract monitored at 280 nm. Peak identification (t_R, min):1, B3 (34.5); 2, B1 (38.4); 3, T2 (40.4); 4, B4 (45.9); 5, B2 (54.8); 6, B2-3-O-gallate (57.0); 7, B2-3′-O-gallate (65.0); 8, B1-3-O-gallate (72.8); 9, C1 (74.0).

individual catechins and PC. The mobile phases for catechins were (A), acetic acid/bidistilled water (2.5:97.5) and (B), acetonitrile/A (80:20). A linear gradient was run from 93 volumes of A + 7 volumes of B to 70 volumes of A + 30 volumes of B during 40 min, followed by another to 100 volumes of B during 5 min, where it was held for 10 min. The mobile phases for the separation of PC were (A) acetic acid/bidistilled water (10:90) and (B) bidistilled water. A linear gradient was run from 10 volumes of A + 90 volumes of B to 70 volumes of A + 30 volumes of B during 45 min, followed by another from 70 volumes of A + 30 volumes of B to 90 volumes of A + 10 volumes of B during 37 min, and then to pure A during 10 min. The flow rate was 1 mL/min for catechins and 0.9 mL/min for PC. Between two injections, the column was washed isocratically, using methanol/water (50:50, v/v), for 15 min, in both methods. All analyses were done in duplicate at least.

RESULTS AND DISCUSSION

Representative chromatograms of the catechins and PC fractions of the grape seed extracts are shown in Figures 1 and 2. The results of the analyses on 17 cultivars are presented in Table 2. To facilitate comparison to literature values based on cluster weight, Table 2 presents data on the contribution of seeds to cluster weight. In the seeds of 10 of the examined 17 cultivars, Cat and Ec were present in higher concentrations than any of the individual PC. Similar observations were reported in the literature (Bourzeix et al., 1986b; Kovac et al., 1990; Dumon et al., 1991; Escribano-Bailón et al., 1992a; Santos-Buelga et al., 1995). The concentration of Ec was higher than that of Cat in 12 cultivars. Romeyer et al. (1986) found this to be the case in half of the examined cultivars while others found that Cat was present in higher concentration than Ec in most cultivars (Bourzeix et al., 1986b; Kontek, 1986; Kovac et al., 1990; Santos-Buelga et al., 1995).

In the seeds of all but three of the examined grape cultivars B2 was the PC present in the highest concentration. The other PC present in relatively large quantities in most cultivars were B4, T2, B1, and B3 but not always following this order of ranking. The galloylated PC were present as minor constituents in most cultivars. Notable exceptions were Gamay, Vincent, and Merlot. Most studies carried out on the PC composition of seeds from ripe grapes (Bourzeix et al., 1986a,b; Romeyer et al., 1986; Kovac et al., 1990; Ricardo da Silva et al., 1991a, 1992a,b; Hmamouchi et al., 1994; Santos-Buelga et al., 1995) found B2 the major PC and the rank order of the other PC variable. The data presented here as well as that in the literature suggest that cultivar has a major influence on the quantitative individual PC composition of grape seeds.

In most cases, the concentrations of individual PC in the seeds were within the ranges reported in the literature (Ricardo da Silva et al., 1991a, 1992a,b; Hmamouchi et al., 1994). About one-third of the PC values, particularly those for B2-3′-O-gallate and C1, were lower than the minima reported in the literature. Bourzeix and co-workers (1986b) reported considerably

Table 2. Catechin and Procyanidin Composition of Seeds of Different Grape Cultivars (mg/100 g)[a]

			catechins		procyanidins[d]									total			
cultivar	col[b]	seed %[c]	(+)-catechin	(−)-epicatechin	B1	B2	B3	B4	B1-3-O-g	B2-3-O-g	B2-3′-O-g	C1	T2	catechins	dimers	dimer gallates	trimers
vinifera																	
Cabernet Franc	R	6.6	96	136	26	79	21	43	24	16	3	tr[e]	26	232	169	43	26
Cabernet Sauvignon[f]	R	4.8	58	67	17	50	9	19	11	tr	tr	2	30	125	95	11	32
Gamay	R	4.4	114	114	62	93	71	149	74	26	8	tr	67	228	375	108	67
Merlot	R	7.2	64	79	20	48	8	21	25	8	4	tr	23	143	97	37	23
Pinot noir	R	5.9	244	193	56	90	30	59	21	9	11	8	76	437	235	41	84
Chardonnay[f]	W	4.9	42	99	25	61	14	26	14	3	tr	6	3	141	126	17	9
Riesling	W	4.9	25	24	11	29	2	12	tr	2	2	tr	7	49	54	4	7
hybrid																	
Baco noir	R	7.2	98	106	33	91	41	127	21	26	7	10	43	204	292	54	53
DeChaunac	R	7.4	135	78	4	22	2	12	tr	tr	4	tr	17	213	40	4	17
Maréchal Foch	R	9.1	46	42	14	43	23	61	11	12	1	4	6	88	141	24	10
Vincent	R	9.3	155	284	60	106	27	45	41	9	4	tr	28	439	238	54	28
Brights 12	R	6.2	23	52	6	24	tr	10	4	3	7	tr	9	75	40	14	9
V65115	R	7.9	47	72	9	18	tr	3	5	tr	2	5	13	119	30	7	18
Seyval	W	7.5	21	23	3	9	2	2	tr	tr	tr	0	tr	44	16	tr	tr
labrusca																	
Concord	R	7.0	37	88	7	75	tr	16	4	4	5	2	8	125	98	13	10
Elvira	W	10.3	40	55	11	29	2	3	3	2	1	1	4	95	45	7	5
Niagara	W	5.2	58	97	8	39	tr	2	tr	2	8	tr	17	155	49	10	17

[a] Monomers were expressed as (+)-catechin equivalents, dimers and trimers as B2 equivalents, and dimer gallates as B2-3′-O-gallate equivalents. [b] Color: R = red; W = white. [c] Contribution of seed weight to cluster weight. [d] B1, B2, B3, B4 = dimers; B1-3-O-g, B2-3-O-g, B2-3′-O-g = dimer gallates; C1, T2 = trimers. [e] Traces. [f] Vintage 1994 while those unmarked were from 1993.

higher concentrations of Cat, Ec, and PC in the fresh seeds of Merlot and Pinot noir than found in this study. The cooler climate of the Niagara region could explain the lower values found in this study.

The data in Table 2 suggests that the quantities of catechins and PC were influenced by genetic factors. In general the seeds of red grape cultivars contained higher quantities of these compounds than the whites. The greater capacity of red cultivars to synthesize phenolic compounds could explain this trend. Among the red cultivars Pinot noir had the highest Cat, T2, B2-3′-O-gallate, and total analyzed flavan-3-ol contents; Vincent, a Vineland hybrid with extremely high anthocyanin concentration (Fuleki, 1990), had the highest Ec, B2, and total catechins; Baco noir (a *Vitis vinifera*−*Vitis riparia* hybrid from France), the highest C1 content. The seeds of Gamay were richest in the other individual PC as well as total PC. Brights 12 and V65115, two highly pigmented hybrids containing only monoglucosidic anthocyanins (Fuleki, 1990), had surprisingly low catechins and PC contents. Bourzeix et al. (1986b) determined the catechins and PC contents in the seeds of 10 grape cultivars and found that Pinot noir was the highest in Cat, Ec, B1, B2, B3, B4, trimer, and tetramer.

Among the white cultivars, Niagara, a labrusca type cultivar used mainly for juice, was the highest in Cat, and Chardonnay in Ec and total PC. The results of this study indicate that, among the examined grape cultivars, the seeds of Gamay, Pinot noir, Baco noir, and Vincent are good sources for the analyzed nutraceutical compounds.

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LITERATURE CITED

Ariga, T.; Hamano, M. Radical scavenging action and its mode in procyanidins B-1 and B-3 from azuki beans to peroxy radicals. *Agric. Biol. Chem.* **1990**, *54*, 2499−2504.

Arpentine, G.; Fernandez, Y.; Bourzeix, M.; Mitjavila, S. Relation between the structure of a series of procyanidins and their radical superoxide scavenger capacity. *Proc. Group Polyphenols* **1992**, *16*, 237−240.

Boukharta, M. Study of *Vitis vinifera* flavonoids: structures of the procyanidins of grape seeds, canes and leaves. Ph.D. Thesis, Institut National Polytechnique de Lorraine, Nancy, France, 1988.

Bourzeix, M.; Clarens, M.; Heredia, N. The procyanidins of grape and wine. *Proc. Group Polyphenols* **1986a**, *13*, 403−411.

Bourzeix, M.; Weyland, D.; Heredia, N. A study of catechins and procyanidins of grape clusters, the wine and other by-products of the wine. *Bull. O.I.V.* **1986b**, *59*, 1171−1254.

Das, N. P. Studies on flavonoid metabolism. *Biochem. Pharmacol.* **1971**, *20*, 2435−2445.

Dumon, M. C.; Michaud, J.; Masquelier, J. Determination of procyanidins of grape seeds of red and white vine varieties from the Bordeaux region. *Bull. O.I.V.* **1991**, *64*, 533−542.

Elstner, E. F.; Kleber, E. Radical scavenger properties of leucocyanidine. In *Flavonoids in Biology & Medicine III: Current Issues in Flavonoid Research*; Das, N. P., Ed.; National University of Singapore: Singapore, 1990; p 227−235.

Escribano-Bailón, T.; Gutiérrez-Fernandez, Y.; Rivas-Gonzalo, J. C.; Santos-Buelga, C. Characterization of procyanidins of *Vitis vinifera* variety Tinta del País grape seeds. *J. Agric. Food Chem.* **1992a**, *40*, 1794−1799.

Escribano-Bailón, T.; Gutiérrez-Fernandez, Y.; Rivas-Gonzalo, J. C.; Santos-Buelga, C. Analysis of flavan-3-ol from *Vitis vinifera* variety Tempranillo grape seeds. *Proc. Group Polyphenols* **1992b**, *16*, 129−132.

Escribano-Bailón, M. T.; Guerra, M. T.; Rivas-Gonzalo, J. C.; Santos-Buelga, C. Proanthocyanidins in skins from different grape varieties. *Z. Lebensm.-Unters.-Forsch.* **1995**, *200*, 221−224.

Frankel, E. N.; Waterhouse, A. L.; Kinsella, J. E. Inhibition of human LDL oxidation by resveratrol. *Lancet* **1993**, *341*,1103−1104.

Fuleki, T. Anthocyanin composition of some highly pigmented grape cultivars. *Proc. Group Polyphenols* **1990**, *15*, 37−40.

Gábor, M. Anti-inflammatory and anti-allergic properties of flavonoids. In *Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological, and Structure-Activity Relationships*; Cody, V., Middleton, E., Harborne, J. B., Eds.; Alan R. Liss, Inc.: New York, 1986; p 471−480.

Hackett, A. M.; Griffiths, L. A.; Broillet, A.; Wermeille, M. The metabolism and excretion of dextro carbon-14 labeled cyanidanol-3 in man following oral administration. *Xenobiotica* **1983**, *13*, 279−283.

Hmamouchi, M.; Es-Safi, N.; Boubekri, C.; Bourzeix, M.; Essassi, E. M. Qualitative and quantitative analysis for cluster proanthocyanidins of four grape varieties issuing from Morocco. *Bull. O.I.V.* **1994**, *67*, 901−915.

Hu, J. P.; Calomme, M.; Lasure, A.; De Bruyne, T.; Pieters, L.; Vlietinck, A.; Vanden Berghe, D. A. Structure-activity relationship of flavonoids with superoxide scavenging activity. *Biol. Trace Elem. Res.* **1995**, *47*, 327−331.

Jimenez-Ramsey, L. M.; Rogler, J. C.; Housley, T. L.; Butler, L. G.; Elkin, R. G. Absorption and distribution of ^{14}C-labeled condensed tannins and related sorghum phenolics in chickens. *J. Agric. Food Chem.* **1994**, *42*, 963−967.

Kontek, A. Determination by HPLC of (+)-catechin and (−)-epicatechin in grapes and wine. *Proc. Group Polyphenols* **1986**, *13*, 467−469.

Kovac, V.; Bourzeix, M.; Heredia, N.; Ramos, T. A study of catechins and procyanidins of white grapes and wine. *Rev. Fr. Oenol.* (*Cah. Sci.*) **1990**, *30*, (125), 7−14.

Lacaille-Dubois, M. A.; Wagner, H. Biological activities of flavonoids: recent developments. *Proc. Group Polyphenols* **1992,** *16*, 217−228.

Laparra, J.; Michaud, J.; Masquelier, J. Pharmacokinetic study of flavanolic oligomers. *Plant. Med. Phytother.* **1977**, *11*, 133−142.

Laparra, J.; Michaud, J.; Masquelier, J. Action of oligomeric procyanidins on vitamin C deficient guinea pig. *Bull. Soc. Pharm. Bordeaux* **1979**, *118*, 7−13.

Lea, A. G. H.; Bridle, P.; Timberlake, C. F.; Singleton, V. The procyanidins of white grapes and wines. *Am. J. Enol. Vitic.* **1979**, *30*, 289−300.

Liu, L.; Castonguay, A. Inhibition of the metabolism and genotoxicity of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in rat hepatocytes by (+)-catechin. *Carcinogenesis* **1991**, *12*, 1203−1208.

Liviero, L.; Puglisi, P. P.; Morazzoni, P.; Bombardelli, E. Antimutagenic activity of procyanidins from *Vitis vinifera*. *Fitoterapia* **1994**, *65*, 203−209.

Maffei Facino, R.; Carini, M.; Aldini, G.; Bombardelli, E.; Morazzoni, P.; Morelli, R. Free radicals scavenging action and anti-enzyme activities of procyanidines from *Vitis vinifera*. *Arzneim.-Forsch./Drug Res.* **1994**, *44*, 592−601.

Mangiapane, H.; Thomson, J.; Salter, A.; Brown, S.; Bell, G. D.; White, D. A. The inhibition of the oxidation of low density lipoprotein by (+)-catechin, a naturally occurring flavonoid. *Biochem. Pharmacol.* **1992**, *43*, 445−450.

Masquelier, J. Wine and atherosclerosis. *C. R. Symp. Int. Aliment.* **1982**, 147−155.

Masquelier, J. Physiological effects of wine. His share in alcoholism. *Bull. O.I.V.* **1988**, *61*, 554−578.

Meunier, M. T.; Villie, F.; Jonadet, M.; Bastide, J.; Bastide, P. Inhibition of angiotensin I converting enzyme by flavanolic compounds: *in-vitro* and *in-vivo* studies. *Planta Med.* **1987**, *53*, 12−15.

Midorikawa, T.; Miura, S.; Hamada, T.; Satomi, O.; Awata, N. Metabolic fate of cianidanol (KB-53). (2) Metabolic fate and effects of cianidanol of hepatic drug metabolizing enzyme system in rats following repeated oral administration. *Pharmacometrics* **1983**, *25*, 1007−1014.

Ozaki, M.; Uchida, S.; Furukawa, K.; Akashi, T; Niwa, M.; Nonaka, G.; Nishioka, I. Effects of condensed tannins on lipid peroxidation in rat brain homogenates and plasma levels of (−)-epigallocatechin 3-O-gallate. In *Flavonoids in Biology & Medicine III: Current Issues in Flavonoid Research*. Das, N. P., Ed.; National University of Singapore: Singapore, 1990; p 259−265.

Perrissoud, D. The development of cianidanol and 3-palmitoyl-(+)-catechin as drugs for the treatment of liver diseases. In *Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological and Structure-Activity Relationships*. Cody, V., Middleton, E., Harborne, J. B., Eds.; Alan R. Liss, Inc.: New York, 1986; p 559−569.

Prieur, C.; Rigaud, J.; Cheynier, V.; Moutounet, M. Oligomeric and polymeric procyanidins from grape seeds. *Phytochemistry* **1994**, *36*, 781−784.

Ricardo da Silva, J. M.; Rosec, J. P.; Bourzeix, M.; Heredia, N. Separation and quantitative determination of grape and wine procyanidins by high performance reversed phase liquid chromatography. *J. Sci. Food Agric.* **1990**, *53*, 85−92.

Ricardo da Silva, J. M.; Bourzeix, M.; Cheynier, V.; Moutounet, M. Procyanidin composition of Chardonnay, Mauzac and Grenache blanc grapes. *Vitis* **1991a**, *30*, 245−252.

Ricardo da Silva, J. M.; Darmon, N.; Fernandez, Y.; Mitjavila, S. Oxygen free radical scavenger capacity in aqueous models of different procyanidins from grape seeds. *J. Agric. Food Chem.* **1991b**, *39*, 1549−1552.

Ricardo da Silva, J. M.; Rigaud, J.; Cheynier, V.; Cheminat, A.; Moutounet, M. Procyanidin dimers and trimers from grape seeds. *Phytochemistry* **1991c**, *30*, 1259−1264.

Ricardo da Silva, J. M.; Belchior, A. P.; Spranger, M. I.; Bourzeix, M. Oligomeric procyanidins of three grapevine varieties and wines from Portugal. *Sci. Aliments* **1992a**, *12*, 223−237.

Ricardo da Silva, J. M.; Rosec, J.-P.; Bourzeix, M.; Mourgues, J.; Moutounet, M. Dimer and trimer procyanidins in Carignan and Mourvédre grapes and red wines. *Vitis* **1992b**, *31*, 55−63.

Romeyer, F. M.; Macheix, J. J.; Sapis, J. C. Changes and importance of oligomeric procyanidins during maturation of grape seeds. *Phytochemistry* **1986**, *25*, 219−221.

Santos-Buelga, C.; Francia-Aricha, E. M.; Escribano-Bailón, M. T. Comparative flavan-3-ol composition of seeds from different grape varieties. *Food Chem.* **1995**, *53*, 197−201.

Scott, B. C.; Butler, J.; Halliwell, B.; Aruoma, O. I. Evaluation of the antioxidant actions of ferulic acid and catechins. *Free Radical. Res. Commun.* **1993**, *19*, 241−253.

Selway, J. W. T. Antiviral activity of flavones and flavans. In *Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological, and Structure-Activity Relationships*; Cody, V., Middleton, E., Harborne, J. B., Eds.; Alan R. Liss, Inc.: New York, 1986; p 521−536.

Su, C. T.; Singleton, V. L. Identification of three flavan-3-ols from grapes. *Phytochemistry* **1969**, *8*, 1553−1558.

Takechi, M.; Tanaka, Y.; Nonaka, G. I.; Nishioka, I. Structure and antiherpetic activity among the tannins. *Phytochemistry* **1985**, *24*, 2245−2250.

Tanahashi, H.; Suwa, Y.; Zenibayashi, Y.; Toyoda, Y.; Kitamura, K.; Hosoda, K.; Amachi, T.; Kondo, K.; Matsumuto, A.; Itakura, H. Clinical and component studies on antioxidant ability of red wine (Abstract). *Am. J. Enol. Vitic.* **1995**, *46*, 405−406.

Teissedre, P. L.; Frankel, E. N.; Waterhouse, A. L.; Peleg, H.; German, J. B. Inhibition of *in vitro* human LDL oxidation by phenolic antioxidants from grapes and wines. *J. Sci. Food Agric.* **1996**, *70*, 55−61.

Terencio, M. C.; Sanz, M. J.; Paya, M. Antihypertensive action of a procyanidin glycoside from *Rhamnus lycioides*. *J. Ethnopharmacol.* **1991**, *31*, 109−114.

Uchida, S.; Edamatsu, R.; Hiramatsu, M.; Mori, A.; Nonaka, G. Y.; Nishioka, I.; Niwa, M.; Ozaki, M. Condensed tannins scavenge active oxygen free radicals. *Med. Sci. Res.* **1987**, *15*, 831−832.

Vinson, J. A.; Dabbagh, Y. A.; Serry, M. M.; Jang, J. Plant flavonoids, especially tea flavonols, are powerful antioxidants using an *in vitro* oxidation model for heart disease. *J. Agric. Food Chem.* **1995**, *43*, 2800−2802.

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