Toward the Fingerprinting of Wines: Cultivar-Related Patterns of Polyphenolic Constituents in Ontario Wines

George J. Soleas,[†,‡] Judy Dam,[§] Michelle Carey,[§] and David M. Goldberg*[,†]

Department of Clinical Biochemistry, Banting Institute, University of Toronto, 100 College Street, Toronto, Ontario M5G 1L5, Canada, Liquor Control Board of Ontario, 55 Lakeshore Boulevard East, Toronto, Ontario M5E 1A4, Canada, and Andres Wines Ltd., 697 South Service Road, Winona, Ontario L8E 5S4, Canada

The concentrations of 15 polyphenols (the phenolic acids gentisic, vanillic, ferulic, *m*-coumaric, *p*-coumaric, caffeic, and gallic acid; the trihydroxystilbenes *cis*- and *trans*-resveratrol and *cis*- and *trans*-polydatin; and the flavonoids, catechin, epicatechin, quercetin, and morin) were measured in wines from a range of white (Chardonnay, Riesling, Seyval Blanc, Vidal) and red (Pinot Noir, Cabernet Sauvignon, Cabernet Franc, Merlot, Gamay Noir) cultivars grown in the viticultural region of Niagara, ON, Canada, to determine whether significant and characteristic differences in the content and relative patterns of individual polyphenols could be identified among the wines of various cultivars. An assay involving solid-phase extraction followed by derivatization and gas chromatography/mass spectrometry was used to quantify the polyphenols. Overlapping values between red and white wines were observed for gentisic, ferulic, *p*-coumaric, and caffeic acid; the concentrations of the other polyphenols were 5−20-fold higher in red wines, apart from *m*-coumaric acid and morin, which could not be detected in any of these wines. Ferulic acid was the highest of all phenolic acids in Riesling wine; in wines from the other three white grape cultivars, caffeic acid and *p*-coumaric acid were the highest, with caffeic acid being the highest in Chardonnay and Vidal wines and *p*-coumaric acid in those from Seyval Blanc. The latter had the lowest mean total phenolic acid concentration (0.035 mmol/L) compared with Chardonnay, Vidal, and Riesling (0.052, 0.066, and 0.075 mmol/L, respectively). The individual phenolic acids demonstrated a similar pattern among all of the red wines analyzed, with gallic acid being the highest and caffeic acid the second highest. Their mean total phenolic acid concentrations spanned a narrow range from 0.200 mmol/L (Gamay Noir) to 0.250 mmol/L (Pinot Noir). Of the hydroxystilbene components, Pinot Noir wines were notable in having by far the highest polydatin concentrations, whereas their concentrations of free resveratrol isomers were among the lowest in this study. Of the flavonoid components, Pinot Noir wines were highest in catechin and epicatechin concentrations, whereas wines from Cabernet Sauvignon that were lowest in the latter two flavonoids had the highest quercetin concentrations.

Keywords: *Phenolic acids; hydroxystilbenes; flavonoids; gas chromatography/mass spectrometry; Vitaceae*

INTRODUCTION

Wine includes a number of components that can profoundly affect human health. Its major constituent, ethanol, has been shown to increase the serum concentration of high-density lipoprotein (HDL) cholesterol (Valimaki *et al*., 1988; Hartung *et al*., 1990), a negative risk factor for coronary heart disease (CHD), and to inhibit platelet aggregation (Rand *et al*., 1988; Veenstra *et al*., 1990), an early phenomenon initiating the endothelial damage that progresses to atherosclerosis as well as a late phenomenon preceding the final occlusion of the artery and the onset of clinical symptoms (Ross, 1993). Between them, these two mechanisms can account for 75−80% of the potential of moderate alcohol consumption to reduce the risk of CHD mortality (Criqui, 1996; Renaud and Ruf, 1996).

Another set of constituents, the polyphenols, which are much more prominent in red wine, display a wide spectrum of effects *in vitro*. Foremost is their role as antioxidants, manifested by their ability to act as free radical scavengers (Yutin *et al*., 1990; Salah *et al*., 1995), to prevent oxidation of low-density lipoproteins (LDL) (Frankel *et al*., 1993, 1995; Teissedre *et al*., 1996), to protect cells against oxidative stress (Nakayama, 1994; Subirade *et al*., 1995), and to elevate total blood antioxidant activity after oral administration (Maxwell *et al*., 1994; Whitehead *et al*., 1995). Other effects include modulation of eicosanoid synthesis toward a more antiatherogenic pattern (Moroney *et al*., 1988; Pace-Asciak *et al*., 1995) and inhibition of tumor growth *in vitro* (Wattenberg *et al*., 1980; Huang *et al*., 1988; Yoshida *et al*., 1990; Uddin and Choudhry, 1995) and, as demonstrated recently, in human cancer patients (Ferry *et al*., 1996). These properties provide a rationale for exploring the polyphenol content of commercial wines to define those that are especially abundant in these desirable compounds and to stimulate the development of enological techniques for their enrichment.

Unlike ethanol, which is a product of yeast metabolism, the synthesis of polyphenols is an intrinsic function of plants that proceeds by means of a number of interrelated biochemical systems including the shikimate, cinnamate, chalcone, and stilbene pathways (Kreuzaler and Hahlbrock, 1975; Fritzemeier and Kindl, 1981; Hrazdina *et al*., 1984; Melchior and Kindl, 1991).

* Author to whom correspondence should be addressed [telephone (416) 978-2638; fax (416) 978-5650].
† University of Toronto.
‡ Liquor Control Board of Ontario.
§ Andres Wines Ltd.

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The synthesis of several of these constituents is modulated by environmental factors such as sunlight (Amerine and Roessler, 1982) and botrytis infection (Jeandet *et al*., 1995), but it is likely that constitutive gene expression is the predominant determining factor for each individual cultivar and that their phenolic patterns, also reflected in the wine produced from the berry must, may demonstrate specific features that can aid their identification. Up to the present, morphological techniques (Galet, 1980) and more recently molecular biological procedures (Bourquin *et al*., 1991; Mauro *et al*., 1992) have been used to characterize individual *Vitis* cultivars, but these are not applicable to the identification of the wines to which they give rise.

A number of publications (Baranowski and Nagel, 1981; Oszmianski *et al*., 1988; Jeandet *et al*., 1993; Frankel *et al*., 1995; Goldberg *et al*., 1995, 1996a,b) have examined the phenolic content of grapes, juices, and wines, often in relation to a single cultivar, but these have tended to include rather few compounds of interest. Many authors have published data on only one sample of each cultivar studied. Little attempt, if any, has been made to compare and contrast their polyphenol content with a view to defining features that may be specific to the grape and to its resulting wine. Development of a method that permits the simultaneous assay of 15 phenolic constituents in wine (Soleas *et al*., 1997) has enabled us to critically examine the phenolic composition of wines from a range of cultivars grown in the uniquely regulated viticulture region of Niagara, southern Ontario.

MATERIALS AND METHODS

Wines. Commercial Ontario wines (number in parentheses) from the 1995 vintage were analyzed from each of the following varietals: (white wines) Chardonnay (11), Reisling (9), Seyval Blanc (4), and Vidal (3 regular and 3 late harvest); (red wines) Pinot Noir (*6*), Cabernet Sauvignon (5), Cabernet Franc (4), Merlot (3), and Gamay Noir (3). The viticulture region of Niagara covers a small flat area of 18 000 acres between the western shore of Lake Ontario and the Niagara Escarpment. Total annual wine grape tonnage was 35 000 in 1995, the year to which this survey was restricted. More than 50% of the wines analyzed came from a single winery. All of those in the region use similar enological techniques. White wines receive minimal skin contact, and only Chardonnay wines are barrel-aged (6−9 months, differences between these extremes being negligible). Bentonite is employed in finning, and filtration is accomplished by the combined use of diatomaceous earth and membrane. All Niagara wines are subjected to strict regulatory control through the Vintners' Quality Alliance (VQA), which lays down criteria to ensure authenticity of labeling and use of the stated cultivars of Ontario origin. The system is approved by the Governments of Canada and the province of Ontario, and the Liquor Control Board of Ontario participates in its enforcement.

When required, samples were kept at 4 °C in an amber vial filled to completion and protected by foil against sunlight. All analyses were completed within 3 days.

Assay Method. A multiresidue derivatization gas chromatographic assay with mass selective detection (GC-MSD) was used for the simultaneous analysis of 15 phenolic constituents as previously described (Soleas *et al*., 1997c).

Instrumentation. A DB-5HT capillary column (J&W Scientific, Folsom, CA; Part J122-5731) with 5% phenyl-substituted methylpolysiloxane nonpolar stationary phase, cross-linked and double-bonded to the capillary wall with excellent thermal stability and low bleed levels, was used. The dimensions of the column were 30 m × 0.25 mm i.d. × 0.10 μm film thickness, and the column was preceded by a 1 m guard column of the same i.d. connected to the column via a chromfit glass connector (Chromatographic Specialties Inc., Brockville, ON).

A Hewlett-Packard (HP) GC-5890 was used for the analysis, equipped with a split/splitless injection port, interfaced to a MSD-5970; the GC/MSD was controlled by an HP Vectra 486/50N PC utilizing MS ChemStation software G1034C (DOS series) and reporting to an HP laserjet IV printer (Hewlett-Packard, Mississauga, ON).

Ultrahigh-purity helium with in-line Supelpure moisture trap and hydrocarbon trap (Supelco Canada, Mississauga, ON) was used as carrier gas. The carrier gas line pressure was set at 60 psi and the column head pressure at 8 psi; the septum purge was at 2.4 mL/min.

GC Temperature Information: injector, 280 °C; detector (transfer line), 320 °C; oven equilibration time, 1.0 min; initial temperature, 80 °C; initial time, 1.0 min. The oven temperature program was as follows:

level	rate (°C/min)	final temp (°C)	final time (min)
1	20.0	250	1.0
2	6.0	300	2.0
3	20.0	320	4.0

Total run time was 25.8 min.

GC Injector Information: injection source, autoinjector; sample washes, 3; sample pumps, 3; sample volume injected, 1 μL; solvent A (acetone) washes, 4; solvent B (pyridine) washes, 4; injection port, splitless with double gooseneck glass insert and gold plated injector seal and a viton O-ring for high temperatures (Hewlett-Packard).

MS Information: acquisition mode, selective ion monitoring (SIM); solvent delay, 7.80 min; electron multiplier voltage (EMV), 1400; EMV offset, 200; resulting EMV, 1600; tune, customized tune with perfluorotributylamine (PFTBA) tuning standard utilizing ions 219, 414, and 502 amu.

Extraction and Derivatization Procedure. Sep-Pak C_8 cartridges (Waters Canada Ltd., Mississauga, ON) were preconditioned with 3 mL of ethyl acetate, 3 mL of 60% absolute ethanol, and 3 mL of deionized water followed by 2 mL of the last-named. Wine samples were diluted with an equal volume of deionized water to bring the alcohol level to approximately 6%, and exactly 1 mL of diluted sample was injected onto the preconditioned Sep-Pak and allowed to drain by gravity flow (3−5 min). A gentle flow of nitrogen was then introduced over the sample with simultaneous gradual suction on a vacuum manifold (−100kPa) for 45 min (Millipore Canada, Mississauga, ON).

The phenolic compounds were extracted by eluting the dry Sep-Pak with 3 mL of ethyl acetate. The eluate was collected in a centrifuge tube previously spiked with fisetin as internal standard at 1.0 mg/L. The extract was then evaporated to dryness on a nitrogen evaporator (Meyer Organomation Associates Inc., S. Berlin, MA). To ensure complete removal of water, 0.5 mL of methylene chloride was added, vortexed, and evaporated to dryness (azeotropic removal of water). Extracts were further dried in an oven at 70 °C for 15 min and derivatized by incubating with 1.0 mL of 1:1 BSTFA/pyridine using vigorous vortexing and incubating at 70 °C for 30 min. Morin (detection limit = 0.31 mg/L) and *m*-coumaric acid (detection limit = 0.05 mg/L) were not measurable in any of the wines analyzed during this study.

Statistics. All data for individual phenolics are given as mean ± SD (mg/L), since only this presentation allows comparison between our results and those of previous authors for the same constituents. However, data for *total phenolic acids* were derived from the sum of the individual phenolic acids as millimoles per liter, since mass units are meaningless when applied to a mixture of compounds of different molecular weights, and in this instance there are no previous data available for comparison.

RESULTS AND DISCUSSION

White Wines. The four white wines showed extremely low to nondetectable levels of *cis*- and *trans*-

polydatin and quercetin (data not presented). These compounds are primarily found in the skins of the grape berries (Creasy and Coffee, 1988; Boulton *et al.*, 1996) and since white grapes do not undergo skin fermentation and the skin contact prior to fermentation is minimal, the low concentrations of the above compounds are expected.

Gentisic Acid. The four regular white wines had mean concentrations of this compound ranging from 0.15 mg/L (Riesling) to 0.38 mg/L (Seyval Blanc), but the late harvest Vidal wine had the exceptionally high mean concentration of 1.07 mg/L, exceeding the content of all white and red wines analyzed (Figure 1A). The difference between the late harvest Vidal and all other white wines was statistically significant ($p = 0.034$). Since the late harvest grapes of this cultivar are gathered approximately 3−4 weeks after the regular grapes, considerable fruit concentration occurs and could explain the enrichment of this compound in the resulting wine must. Longer sun exposure and maturation may also be explanations.

Vanillic Acid. All regular white wines had low mean concentrations of this compound ranging from 0.09 mg/L (Vidal and Seyval Blanc) to 0.21 mg/L (Riesling). The late harvest Vidal wine once again had the highest mean concentration (0.38 mg/L) among all white wines (Figure 1B), which may be due to the factors proposed above. The difference between the late harvest Vidal and all other white wines analyzed was statistically significant ($p = 0.012$). Contrary to the view that vanillic acid is extracted in wine from oak (Ribéreau-Gayon, 1964), several of our Chardonnay wines that did not receive oak treatment had concentrations of vanillic acid as high as or higher than those that did (data not shown).

Ferulic Acid. The mean concentration of this compound was significantly higher in Riesling wines than in all other regular white wines analyzed ($p < 0.001$), although the late harvest Vidal had even higher concentrations, exceeding those of the red wines in this survey (Figure 1C). The difference between Riesling and late harvest Vidal was not statistically significant ($p > 0.05$). The high concentration of ferulic acid in Riesling wines (4.42 mg/L) seems to be characteristic of this cultivar (*vide infra*).

p-Coumaric Acid. The mean concentration of this compound in white wines ranged from 1.57 mg/L (Chardonnay) to 3.21 mg/L (late harvest Vidal) (Figure 1D). Chardonnay wine was significantly lower in *p*-coumaric acid concentration than all other white wines analyzed ($p = 0.015$), as well as the red wines. The regular Vidal wine (3.01 mg/L) had a concentration of *p*-coumaric acid very similar to that of the late harvest Vidal wine (3.21 mg/L).

Caffeic Acid. The mean concentrations of caffeic acid in Chardonnay, Vidal, and Riesling wines, 5.20, 4.61, and 3.79 mg/L, respectively, were significantly higher ($p = 0.003$) than that of Seyval Blanc at 1.51 mg/L (Figure 1E). The superiority of late harvest Vidal wine seen with the previous four compounds was not observed with caffeic acid. In fact, its concentration in regular Vidal wine was significantly higher than in the late harvest Vidal ($p = 0.013$).

Gallic Acid. The mean concentration of this compound in regular Vidal wine (2.80 mg/L) was the highest ($p = 0.039$) of all white wines analyzed, including the late harvest of this varietal (Figure 1F). All other white wines had similar concentrations of gallic acid.

cis- and trans-Resveratrol. The mean concentrations of *cis*-resveratrol in wines from the various white cultivars were <0.10 mg/L and those of *trans*-resveratrol <0.26 mg/L (Figure 1G,H). Regular Vidal and Seyval Blanc wines, both made from grapes of French hybrid cultivars, had very low to nondetectable concentrations of both resveratrol isomers, but the late harvest Vidal had concentrations for both isomers that were in the same range as those of the two white wines made from the *Vitis vinifera* cultivars Chardonnay and Riesling.

Catechin and Epicatechin. Catechin and epicatechin were present in mean concentrations ranging from 3.80 to 4.20 mg/L and from 1.70 to 3.80 mg/L, respectively (Figure 1I,J). Catechin and epicatechin are found only in the skins, seeds, and stems of grapes (Ribéreau-Gayon, 1964). Since the contact time of white grape skins with the must is minimal, little extraction of these compounds takes place; thus, their narrow ranges and considerably lower concentrations than those of red wines were expected. This also explains why the "concentration" phenomenon observed with the first three constituents is not seen with catechin and epicatechin in late harvest Vidal wine (Figure 1I,J). Regular Vidal wine was significantly higher in its mean catechin concentration than all other white wines analyzed ($p < 0.001$), including late harvest Vidal ($p = 0.018$), and significantly higher in its mean epicatechin concentration than the last mentioned wine ($p = 0.028$) (Figure 1J) but not significantly different from the other white wines with respect to epicatechin. Two possible explanations for the significantly lower concentrations of catechin and epicatechin in late harvest Vidal may be their polymerization to procyanidins due to (a) continuing UV exposure or (b) overmaturation of the grapes.

Red Wines. *Gentisic Acid.* All red wines analyzed had similar mean concentrations of this compound (0.44−0.46 mg/L). Generally, its concentration was twice as high in red than white wines (Figure 1A), excluding late harvest Vidal wine ($p < 0.001$). This suggests that it is likely found in approximately equal amounts in the skins and pulp of the grape and that more of this compound is extracted in red wine due to the skin fermentation. Its higher concentration in late harvest Vidal wine is probably due to the enrichment ("concentration") effect as mentioned previously.

Vanillic Acid. This compound was found in mean concentrations ranging from 2.3 mg/L (Gamay Noir) to 3.7 mg/L (Pinot Noir) (Figure 1B). Gamay Noir had a significantly lower concentration than all other red wines analyzed ($p = 0.012$), possibly due to the minimal skin contact time during fermentation typical of this cultivar. By contrast, Pinot Noir, Cabernet Sauvignon, Cabernet Franc, and Merlot are skin fermented for 10−20 days. The fact that some vanillic acid was found in white wines, which do not receive skin fermentation or, in most cases wood aging, indicates that the skins and pulp are its major sources and probably only a small contribution is made by the oak wood, contrary to what has been previously reported (Ribéreau-Gayon, 1964).

Ferulic Acid. All red wines contained <1.0 mg/L of ferulic acid except Cabernet Franc, which had a mean concentration of 2.86 mg/L. This concentration for Cabernet Franc was much higher than that of all other red wines analyzed, although this difference was not significant, due to large variation in ferulic acid content among Cabernet Franc wines. Likewise, no significant difference was observed ($p > 0.05$) between Cabernet

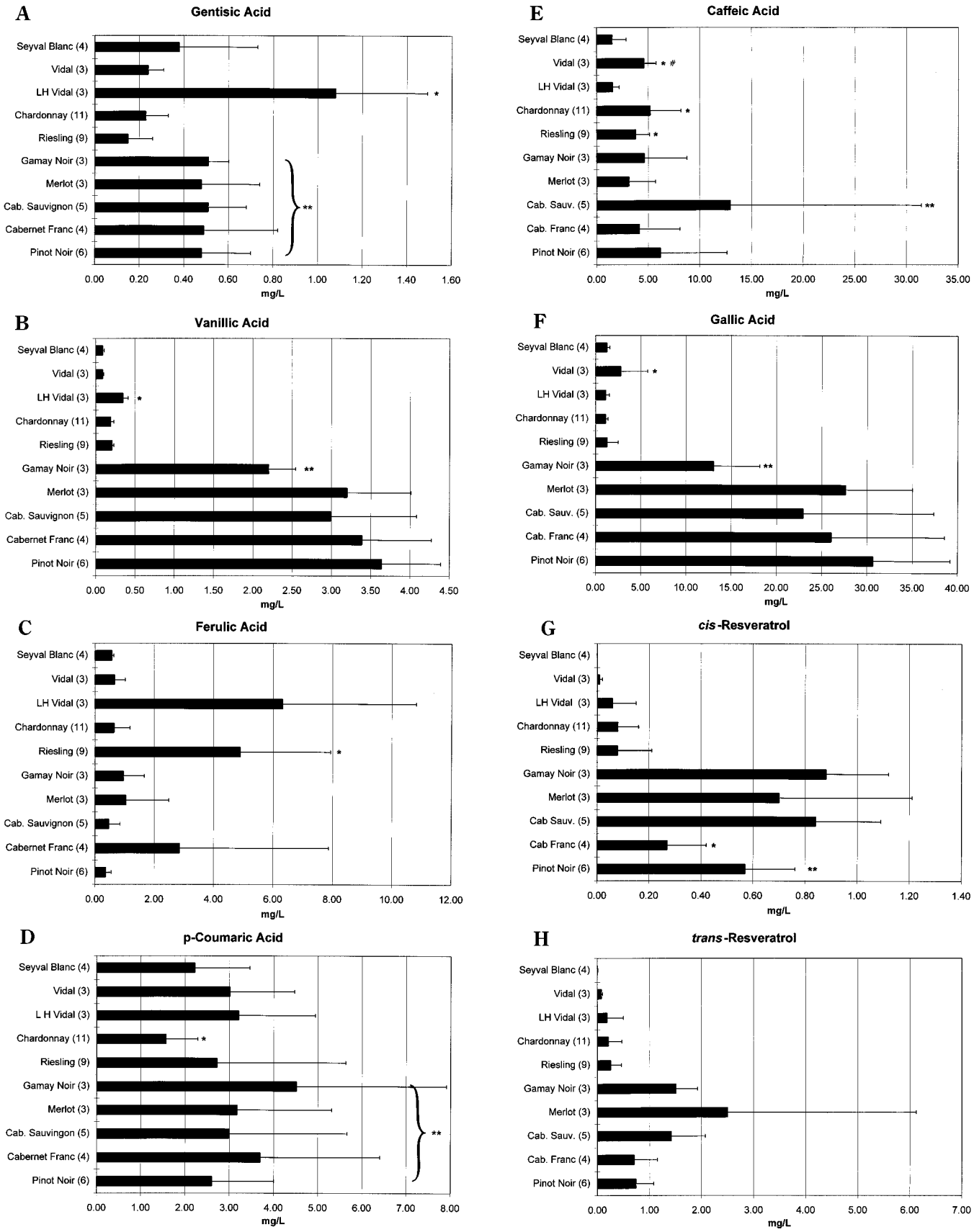
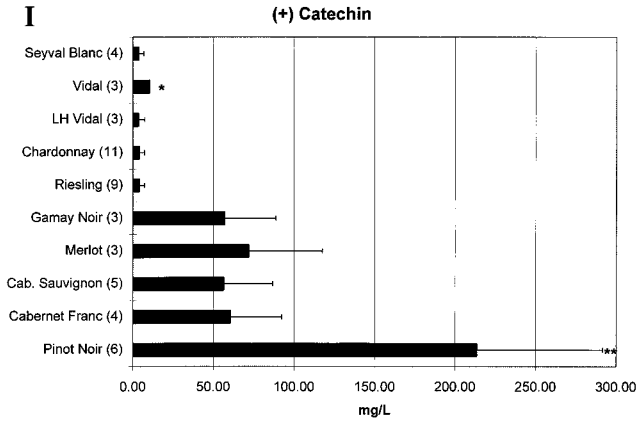
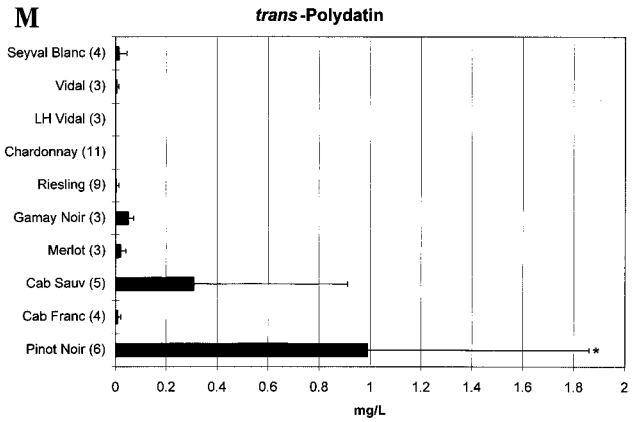
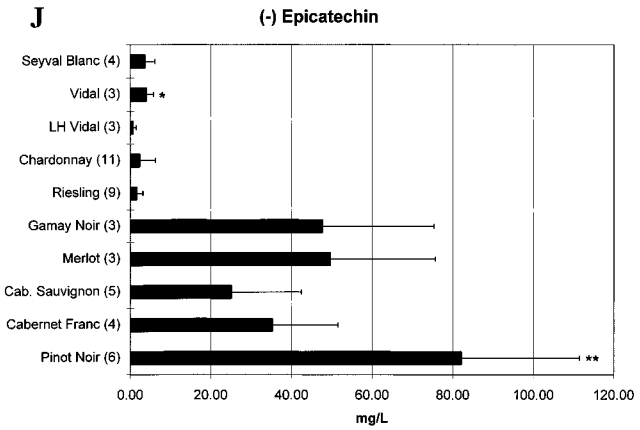
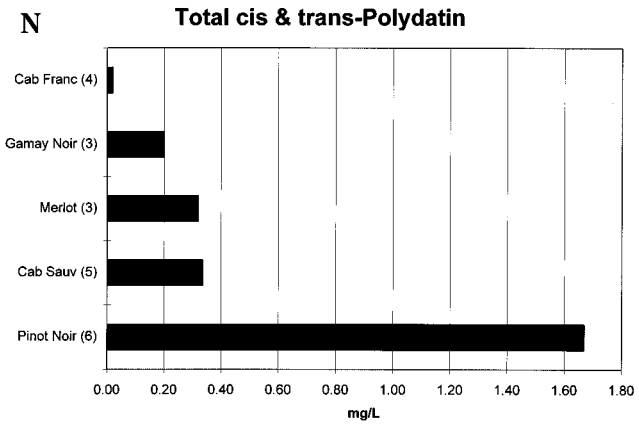


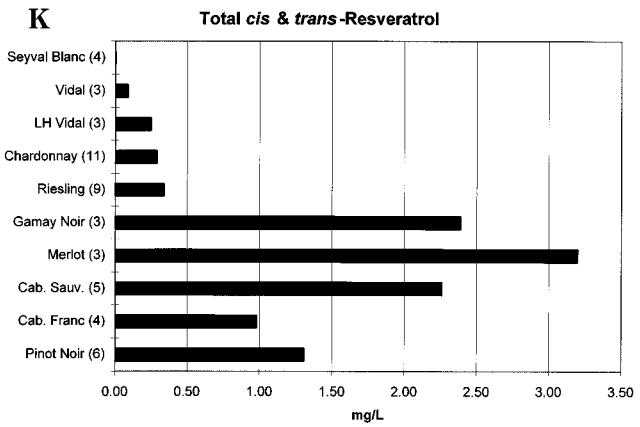
Figure 1. Mean concentrations of polyphenols in white and red wines: (A) gentisic acid [*, significantly higher than all other white wines combined ($p = 0.034$); **, significantly higher than all white wines excluding the late harvest Vidal ($p = 0.001$)]; (B) vanillic acid [*, significantly higher than all other white wines combined ($p = 0.012$); **, significantly lower than all other red wines combined ($p = 0.012$)]; (C) ferulic acid [*, significantly higher than all other regular white wines combined ($p = 0.001$)]; (D) *p*-coumaric acid [*, significantly lower than all other white wines combined ($p = 0.015$); **, significantly higher than all white wines combined ($p = 0.035$)]; (E) caffeic acid [*, significantly higher than Seyval Blanc wine ($p = 0.003$); *#, significantly higher than LH Vidal wine ($p = 0.013$); **, significantly higher than all other red wines combined ($p = 0.042$)]; (F) gallic acid [*, significantly higher than all other white wines combined ($p = 0.039$); **, significantly lower than all other red wines combined ($p = 0.011$)];

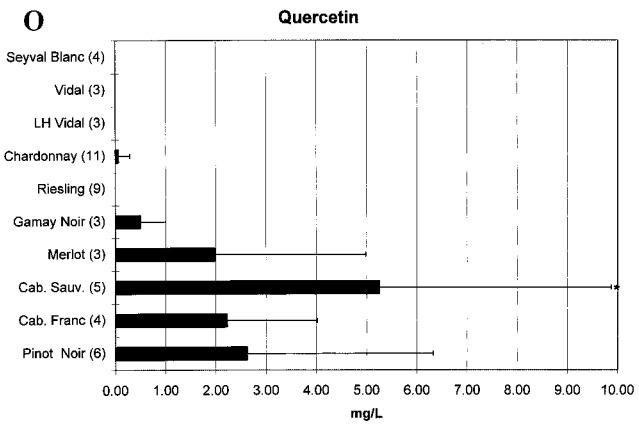


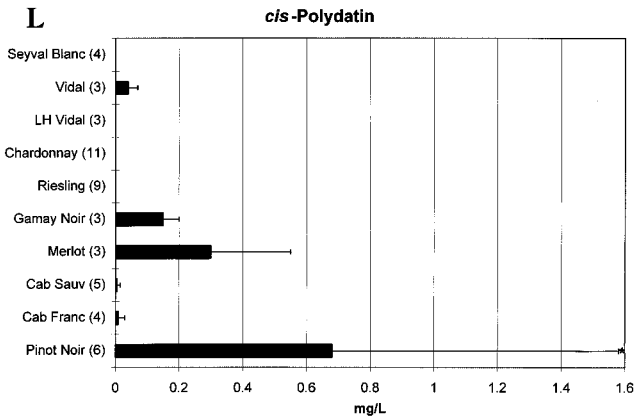












(G) *cis*-resveratrol [*, significantly lower than all other red wines combined ($p = 0.048$); **, significantly lower than Cabernet Sauvignon wines ($p = 0.042$) but not those of Merlot and Gamay Noir]; (H) *trans*-resveratrol; (I) catechin [*, significantly higher than all other white wines combined ($p < 0.001$); **, significantly higher than all other red wines combined ($p < 0.001$)]; (J) epicatechin [*, significantly higher than LH Vidal wine ($p = 0.028$); **, significantly higher than all other red wines combined ($p < 0.003$)]; (K) total *cis*- and *trans*-resveratrol; (L) *cis*-polydatin (red wine only) [*, significantly higher than all other red wines combined ($p < 0.001$)]; (M) *trans*-polydatin (red wine only) [*, significantly higher than all other red wines combined ($p < 0.001$)]; (N) total *cis*- and *trans*-polydatin (red wine only); (O) quercetin [*, significantly higher than all other red wines combined ($p = 0.040$)]. Number of individual samples analyzed for each varietal is in parentheses, all being from entirely different batches.

Franc and all of the white wines analyzed (Figure 1C). The variation between red and white wines was also not significant ($p > 0.05$).

p-Coumaric Acid. Pinot Noir wine had the lowest mean concentration of this compound (2.61 mg/L) with Gamay Noir at the highest (4.5 mg/L). Much like ferulic acid, there was considerable overlap in its concentrations between the two classes of wines (Figure 1D) but the overall ratio between white and red wine was rougly 2:3 ($p = 0.035$). Since it appears that fermentation with the skins present increases the values for *p*-coumaric acid in the resulting wine only by 50%, it is likely that the total amount present in the berry pulp approximates that of the skins from the same berries.

Caffeic Acid. The mean concentrations of this compound in the red wines analyzed ranged from 3.15 mg/L (Merlot) to 12.95 mg/L (Cabernet Sauvignon), and the latter value was signficantly higher than that of all other red wines analyzed ($p = 0.042$) (Figure 1E), a difference that is probably cultivar-dependent.

Gallic Acid. With the exception of Gamay Noir (13.08 mg/L), all other red wines had mean concentrations of this compound >20 mg/L (Figure 1F), with Pinot Noir having the highest (30.67 mg/L). The significantly lower concentration of gallic acid in Gamay Noir compared to that of other red wines analyzed ($p = 0.011$) may be due to the brief skin fermentation time this grape receives relative to other red wines.

cis- and trans-Resveratrol. *cis*-Resveratrol was found in mean concentrations ranging from 0.27 mg/L (Cabernet Franc) to 0.88 mg/L (Gamay Noir) (Figure 1G) and *trans*-resveratrol at higher mean concentrations ranging from 0.71 mg/L (Cabernet Franc) to 2.50 mg/L (Merlot) (Figure 1H). With all cultivars the concentration of *trans*-resveratrol (Figure 1H) was higher than that of the *cis*-isomer (Figure 1G). The mean *cis*-resveratrol concentration of Cabernet Franc was significantly lower than that of all other red wines analyzed ($p = 0.048$) except Pinot Noir. Pinot Noir wines were significantly lower in mean *cis*-resveratrol concentration than were Cabernet Sauvignon wines ($p = 0.042$) but not Merlot wines. Even though the mean *trans*-resveratrol concentration of Merlot wine (2.50 mg/L) was the highest of all red wines (Figure 1H), the difference was not significant ($p > 0.05$).

The high concentrations of *cis*- and *trans*-resveratrol in Gamay Noir relative to the other red wines was unexpected because it is a component of the skins and stems (Creasy and Coffee, 1988) and Gamay Noir receives the least time in skin fermentation. A comparison of these results with wines from the Beaujolais region of France, which are also made from Gamay Noir grapes, may help to explain this phenomenon.

The mean concentrations of total resveratrol isomers ranged between 0.98 mg/L (Cabernet Franc) and 3.20 mg/L (Merlot), following the same pattern as *trans*-resveratrol (Figure 1K).

cis- and trans-Polydatin. *cis*-Polydatin was detected in mean concentrations ranging from 0.02 mg/L (Cabernet Sauvignon) to 0.68 mg/L (Pinot Noir) (Figure 1L) and of *trans*-polydatin in a range between 0.02 mg/L (Cabernet Franc) and 0.98 mg/L (Pinot Noir) (Figure 1M). The concentrations of *cis*- and *trans*-polydatins in Pinot Noir were significantly higher than in all other red wines analyzed ($p < 0.001$ for both isomers). The very low mean concentrations of both polydatin isomers found in wines made from Cabernet Franc were consistent with those of *cis*- and *trans*-resveratrol described above and are probably cultivar-related. The mean concentrations of total polydatin isomers ranged between 0.04 mg/L (Cabernet Franc) and 1.68 mg/L (Pinot Noir) (Figure 1N). The higher concentration of these compounds in Pinot Noir may also be cultivar-related. However, the grapes of this cultivar are harvested much earlier than grapes of other red cultivars, closer to veraison; therefore, shorter UV exposure and earlier maturation may be significant factors in this phenomenon.

Catechin and Epicatechin. Pinot Noir wine had by far the highest mean concentrations of catechin and epicatechin at 213 and 82 mg/L, respectively (Figure 1I,J), and these values were significantly different for both compounds from all other red wines analyzed ($p < 0.001$ and $p < 0.003$, respectively). In all cultivars the mean concentration of catechin was higher than that of epicatechin. Gamay Noir wine exhibited the lowest ratio of catechin to epicatechin at 1.2, whereas Pinot Noir wines had the highest at 2.6.

Quercetin. Cabernet Sauvignon had a significantly higher mean concentration of quercetin at 5.26 mg/L (Figure 1O) than all other red wines analyzed ($p = 0.005$), the mean concentrations of which ranged from 0.50 mg/L (Gamay Noir) to 2.60 mg/L (Pinot Noir). The grapes of this cultivar are harvested much later in the season than most other grapes and are therefore exposed to sunlight for a longer period. Thus, these results are consistent with the notion that exposure of the berries to sunlight may play an important role in determining the quercetin concentrations of red wines (Karumanchiri *et al.*, 1996), as first demonstrated by Price *et al.* (1995) for Pinot Noir.

Phenolic Acid Pattern. A series of interesting patterns was observed in the phenolic acid content of the white and red wines analyzed (Figure 2). Ferulic acid was the highest of all phenolic acids in Riesling wine. In the other three white wine cultivars caffeic acid and *p*-coumaric acid were the highest, with caffeic acid being the highest in Vidal and Chardonnay and *p*-coumaric in Seyval Blanc wines. The order vanillic < gentisic < ferulic < gallic acid was consistently observed for wines from these three cultivars. Thus, the distinctive feature of Riesling was that ferulic acid was the highest of its phenolic constituents and that of Seyval Blanc was that *p*-coumaric acid was the highest. This last point receives emphasis if one notes that caffeic acid exceeded *p*-coumaric acid also in wine from Riesling grapes.

Seyval Blanc had the lowest mean total phenolic acid concentration (this represents the sum of all six phenolic acids expressed in mmol/L) of 0.035 mmol/L, whereas those of Chardonnay, Vidal, and Riesling were higher: 0.052, 0.066, and 0.075 mmol/L, respectively ($p < 0.005$). Since Seyval Blanc grapes reach maturity and are harvested 3−4 weeks prior to the other three cultivars surveyed, it is likely that this phenomenon is related to UV exposure, but genetic cultivar-related factors cannot be ruled out at this time.

The relative orders of the individual phenolic acids among Cabernet Sauvignon, Pinot Noir, Cabernet Franc, Merlot, and Gamay Noir (Figure 2B) wines were essentially similar. All red wines had much higher mean concentrations of gallic acid than of any other phenolic acid. Caffeic acid was the second highest in all of these wines but was significantly higher than the next two (*p*-coumaric and vanillic acids) only in Cabernet Sauvignon wines ($p < 0.050$).

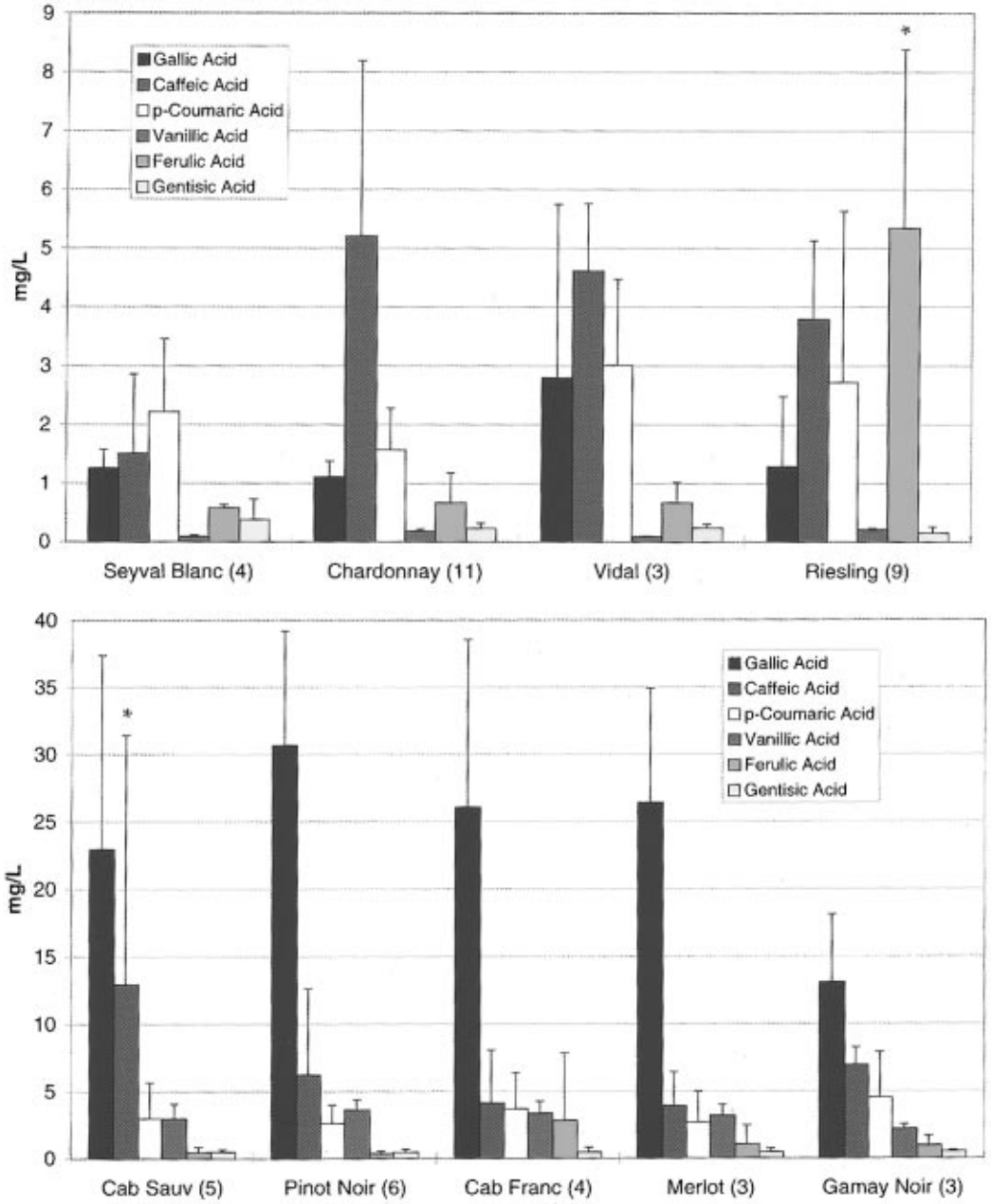


Figure 2. Comparison of phenolic acid mean concentrations for each individual white wine varietal: (A, top) white wines [*, significantly higher than all other white wines ($p = 0.001$)]; (B, bottom) red wines [*, significantly higher than *p*-coumaric and vanillic acid only in Cabernet Sauvignon wines ($p < 0.050$)].

Wines made from all of the red wine cultivars had very similar total phenolic acid concentrations (calculated as described above): Cabernet Sauvignon, 0.249 mmol/L; Pinot Noir, 0.259 mmol/L; Cabernet Franc, 0.237 mmol/L; Merlot, 0.224 mmol/L; Gamay Noir, 0.200 mmol/L.

Comparison of Present Data with Published Values for Wine Polyphenols. The present body of data surpasses the knowledge about the phenolic composition of wines from individual cultivars accumulated up to this time. Data were presented in a manner to accomplish two fundamental objectives: to compare the concentration of each polyphenol in wines from the different cultivars and to examine the pattern of all six phenolic acids in wines from each cultivar. More data have been published on the concentrations of resveratrol isomers and glucosides in wines than of any other phenolic constituent, but as these have been recently reviewed (Soleas *et al*., 1997a), the process will not now be repeated. We have also extensively described the environmental and enological factors that affect wine polyphenol concentrations (Soleas *et al*., 1997b), but by restricting this investigation to wines of a single vintage from a tiny viticultural region with uniform production techniques, we have been able to focus upon the characteristics of the individual varietal wines as unique products of their respective cultivars that in this scenario are probably reflecting true genetic features without distortion due to environmental and enological factors.

Qualitative data only were presented by Wulf and Nagel (1979) for 20 anthocyanin peaks of Cabernet Sauvignon and Pinot Noir grapes (one sample of each); by Baranowski and Nagel (1981) for some phenolic acids of a single Reisling wine; by Lunte (1987), who developed an HPLC procedure to assay flavonoid compounds in wine and grape juice; and by Oszmianski *et al*. (1988), who examined the phenolic constituents of a single wine

from Syrah grapes. In an early quantitative study, Singleton and Trousdale (1983) measured the concentrations of seven phenolics in skins, pomace, and wine from one batch of four white grape cultivars (Chenin Blanc, Semillon, French Colombard, and Thompson Seedless) in two consecutive vintages. French Colombard had the highest catechin and Chenin Blanc the highest epicatechin content in the wines of both vintages, while gallic acid showed rather low and inconsistent concentrations in all the wines. Some astonishing differences in extraction efficiency among the different polyphenols and cultivars were revealed. In French Colombard, the epicatechin content of wine (3.4 mg/L) was only 1% of that of the skins in 1980 but at 25.5 mg/L was 2-fold that of the skins in 1981; the catechin contents of the same wines (around 32−35 mg/L) were relatively similar over the two vintages. These concentrations were much higher than in any of the white wines we examined, but the gallic acid concentrations, as in our study, were around 1 mg/L.

Salagoity-Auguste and Bertrand (1984) presented mean data for Bordeaux red wines (3 samples of each) from Cabernet Sauvignon, Merlot, and Malbec (1982 vintage) of 18 different phenolics including catechin, epicatechin, quercetin, and gallic, vanillic, caffeic, *p*-coumaric, and ferulic acid. In all wines, gallic acid was the highest of the phenolic acids, and their relative orders in wines from Cabernet Sauvignon and Merlot were similar to our own findings. The concentrations of catechin and epicatechin in the Bordeaux wines were 20−40% of the mean values for Canadian Cabernet Sauvignon and Merlot wines, both being around 11 mg/L in Bordeaux Merlot, but the concentrations of quercetin were double the mean values for wines from the respective Canadian cultivars.

Achilli *et al.* (1993) measured up to 36 phenolic constituents in one sample of each of two white and two red monovarietal Italian wines. Gallic acid was extremely high in all (52.6−480.9 mg/L), whereas quercetin was not detected in any. Barbera contained as much as 135 mg/L vanillic acid but contained no detectable catechin, although this was found at levels of 16.4 mg/L (Orvieto) and 7.2 mg/L (Gewurztraminer) in the two white wines. Rather sparse data were published for one Pinot Noir wine [catechin = 110 mg/L and epicatechin = 38 mg/L (Da Silva *et al.*, 1990)] and for the following grapes or juices: Niagara, one sample (Jaworski and Lee, 1987); Concord and De Chaunac, one sample of each (Oszmianski and Lee, 1990); and Thompson Seedless (Spanos and Wrolstad, 1990). Against the background provided by these reports, the wide variance in phenolic composition both between and within the different cultivars and the high SDs noted by us for certain constituents can be better understood.

The most recent survey of wine phenolics was published by Frankel *et al.* (1995) and included 14 red and 6 white Californian wines, in all of which 9 constituents were measured. They presented the data for the individual wines and did not generate mean values for specific cultivars. Overall, it appears that their results were comparable to ours with respect to caffeic acid and quercetin for those cultivars common to both studies, but their reported concentrations for catechin and epicatechin were 2-fold higher and their concentrations for gallic acid about 4 times higher than in the present survey. As with the earlier papers described above, it remains to be established whether differences between this and the present study reflect the diverse methods utilized or altered metabolic behavior of the same cultivars caused by different climatic and environmental conditions. We hope to resolve these questions in an investigation we have initiated to chart the polyphenol concentrations of wines from individual cultivars derived from all of the principal wine-producing regions of the world.

ABBREVIATIONS USED

HDL, high-density lipoprotein; CHD, coronary heart disease; GC-MSD, gas chromatography-mass selective detection; LH, late harvest; LDL, low-density lipoprotein; PFTBA, perfluorotributylamine; BSTFA, bis(trimethylsilyl)trifluoroacetamide; SD, standard deviation; UV, ultraviolet; HPLC, high-performance liquid chromatography.

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