Survey of the Free and Conjugated Myricetin and Quercetin Content of Red Wines of Different Geographical Origins

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Gradient reversed-phase HPLC was used to obtain quantitative estimates of the levels of free and conjugated myricetin and quercetin in 65 red wines from Italy, Chile, France, California, Australia, Bulgaria, Spain, Romania, New Zealand, Brazil, Morocco, and Hungary. The concentrations of total flavonols ranged from 4.6 to 41.6 mg L^{-1}. High total flavonol levels appear to be associated with the use of thick-skinned grape varieties, such as Cabernet Sauvignon, with a high skin:volume ratio, which were left to ripen fully in sunny conditions before harvest and which were extracted efficiently by modern methods of vinification. Some Chilean Cabernet Sauvignon wines contained up to 40 mg of total flavonols L^{-1}, which was higher than the levels detected in Cabernet Sauvignon from France, California, and Australia. The flavonol content of 1989 and 1990 Cabernet Sauvignon from Bulgaria was <6 mg L^{-1}. Chilean Cabernet Sauvignon, Merlot, and Pinot Noir all contained consistently higher concentrations of flavonols than their counterparts from different geographical regions.

Keywords: *HPLC; quantitative analysis; flavonols; myricetin; quercetin; red wines*

INTRODUCTION

Flavonoids are secondary metabolites found ubiquitously in plants (Harborne, 1994), where they function as UV−B protectants [see Koes et al. (1994)] and are involved in the regulation of pollen tube growth in the stigma (Mo et al., 1992; Vogt et al., 1994). They also act as regulatory signals in the transcription of nodulation genes in *Rhizobium* cells as a first step toward legume root nodule formation and symbiotic nitrogen fixation (Firmin et al., 1986). The metal-chelating capability of flavonoids, together with their free-radical-scavenging properties, has led to proposals that flavonoids act as dietary antioxidants (Bors and Saran, 1987). It is this role, as potential inhibitors of free-radical-mediated diseases, such as coronary heart disease, stroke, and cancers, and the associated health benefits of a high-flavonoid diet, which has received the most attention in recent literature.

Flavonoids are phenolic compounds which occur primarily as glycosides (Markham, 1989). They are classified into two major groups: the anthocyanins and the anthoxanthins. The anthoxanthins are subdivided into the flavones, flavanones, and flavonols (Herrmann, 1988). Flavonols, such as quercetin (**I**; Chart 1), myricetin (**II**), isorhamnetin (**III**), and kaempferol (**IV**), and the corresponding flavones, apigenin (**V**), and luteolin (**VI**), have antioxidant properties (Shahidi and Wanasundara, 1992), and their consumption has been associated with a reduced risk of cancer (Verma et al., 1988; Wattenburg, 1985, 1990; Wei et al., 1990), thrombosis (Gryglewski et al., 1987; Laughton et al., 1991), and cardiovascular disease (Gregory et al., 1990; Hertog, 1994). Fruits, vegetables, and beverages are important dietary sources of flavonoids, especially flavonols (Hertog et al., 1992a, 1993b; Crozier et al., 1997b) with beverages accounting for at least 25−30% of the total daily intake (Hertog et al., 1993b). Recent epidemiological studies have suggested that flavonol-rich diets are associated with higher life expectancy (Hertog et al., 1993a, 1995; Hertog and Hollman, 1996).

Red wines are a rich source of flavonoids (Revilla et al., 1986; Hertog et al., 1993b; Frankel et al., 1996), and studies have shown that moderate consumption is associated with a reduced risk of coronary heart disease (Stampfer et al., 1988; Gronbaek et al., 1995). In many countries a high intake of saturated fats has been linked to elevated levels of coronary heart disease, yet this relationship does not apply to certain regions of France, where red wine is consumed in preference to beer, a phenomenon popularly referred to as the “French paradox” (Renaud and Lorgeril, 1992; Renaud, 1996). Maxwell et al. (1994) reported a rapid increase in the antioxidant activity in the serum of 10 human volunteers after drinking red Bordeaux wine. Further studies on this observation have indicated that it is the phenolic components in red wine, rather than the alcohol content, which exert the protective effects (Sato et al., 1996). The phenolics in red wines inhibit the oxidation and cytotoxicity of low-density lipoproteins in vitro which may decrease their artheoreogenicity and explain the re-

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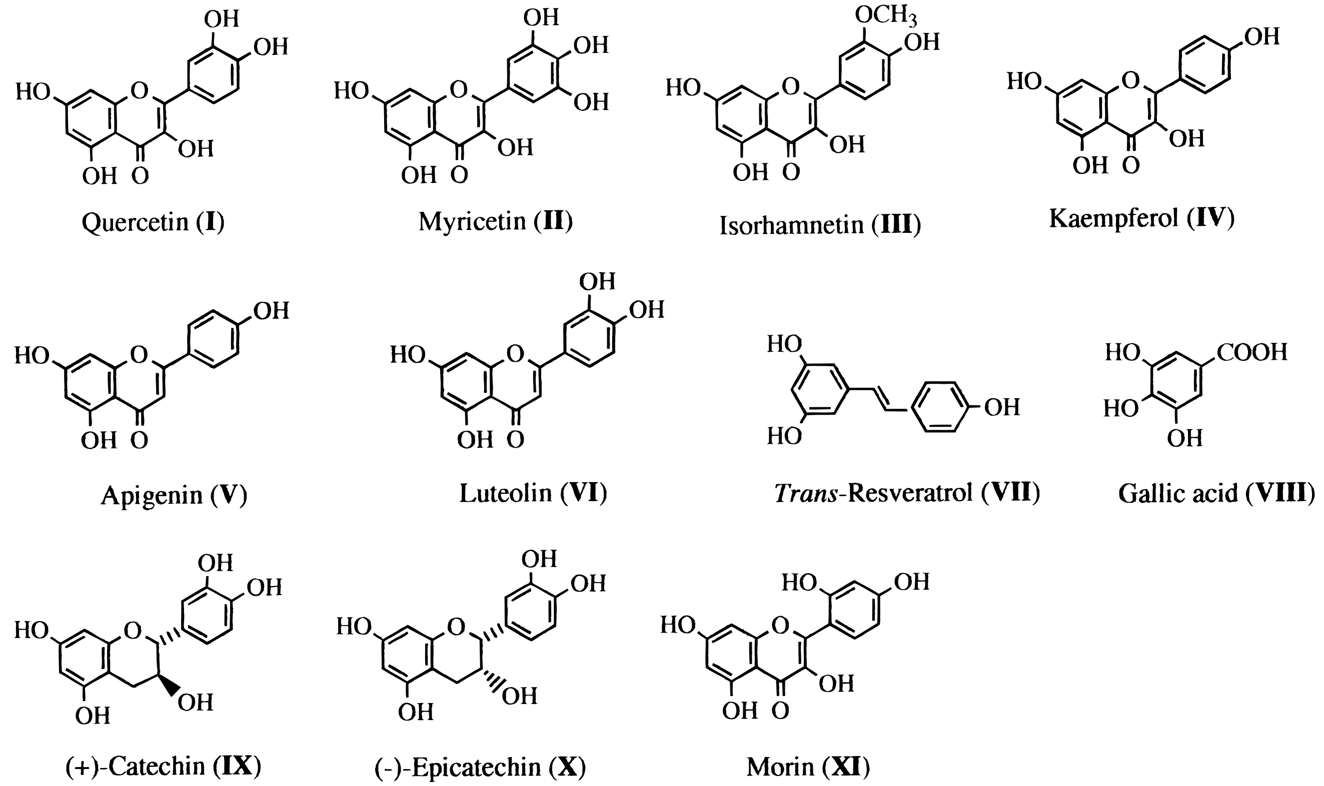
Chart 1. Structures of Quercetin, Myricetin, Isorhamnetin, Kaempferol, Apigenin, Luteolin, *trans*-Resveratrol, Gallic Acid, (+)-Catechin, (−)-Epicatechin, and Morin



duced risk of coronary heart disease (Frankel et al., 1993a, 1995).

One non-flavonoid phenolic that is found in red wine, the stilbene resveratrol (**VII**), has been the focus of interest in the popular press (Jones, 1995; McWhirter, 1996) because of its ability to prevent platelet aggregation in coronary arteries (Kimura et al., 1985) and lessen the incidence of cancer (Jang et al., 1997). However, the hydroxybenzoate gallic acid (**VIII**) and certain flavonoids, most notably the flavan-3-ols (+)-catechin (**IX**) and (−)-epicatechin (**X**), as well as the flavonols quercetin and myricetin, are present in red wines in far higher concentrations (Frankel et al., 1995; Frankel, 1996; Lamuela-Raventós et al., 1995; Goldberg et al., 1995) and exhibit similar, if not greater, antioxidant and antiplatelet aggregation activity than resveratrol [see Frankel et al. (1993a,b, 1995), Vinson et al. (1995), Teissedre et al. (1996)].

The present study, which utilized improved high-performance liquid chromatography (HPLC) procedures (Crozier et al., 1997a) to investigate the flavonol content of 65 red wines, of wide-ranging geographical origin, demonstrates that the wines exhibit marked and systemic differences in the levels of both free and conjugated myricetin and quercetin.

METHODS

Wines. Sixty-five bottles of red wine were purchased from Allders Duty Free Shop, Glasgow Airport, Abbottsinch, Paisley, U.K.; Safeway plc, 373 Byers Rd., Glasgow, U.K.; Oddbins, 181 Byers Rd., Glasgow G12, U.K; J. Sainsbury's plc, 10 Darnley Mains Rd., Glasgow G41, U.K.; Peckham & Rye, 21 Clarence Dr., Glasgow G12, U.K.; Victoria Wine Cellars, 159 Hyndland Rd., Glasgow, U.K.; Tesco Stores Ltd., Telegraph Rd., Heswall, The Wirral, Cheshire, U.K.

Extraction and Hydrolysis Conditions. Optimization of acidic conditions for the hydrolysis of flavonol conjugates in wines has been described by Hertog et al. (1992b) following an earlier detailed study by Harborne (1965) on the release of free flavonoids by acid and enzymic hydrolyses. In the present investigation, preliminary screenings were carried out to ascertain the most effective acid hydrolysis conditions. As a result, all samples were hydrolyzed at 90 °C for 2 h in 1.2 M HCl in 50% aqueous methanol, containing morin (**XI**) as an internal standard and 20 mM sodium diethyldithiocarbamate as an antioxidant. A microscale hydrolysis procedure was used which involved adding a 300-μL sample of wine to a 3-mL glass V-vial together with 400 μL of 6 M HCl, 300 μL of distilled water, and 1 mL of methanol containing 40 mM sodium diethyldithiocarbamate and 5 μg of morin. A Teflon-coated magnetic stirrer was placed in the vial which was sealed tightly with a PTFE-faced septum prior to heating in a Reacti-Therm heating/stirring module (Pierce, Rockford, IL). Extract aliquots of 100 μL, taken both before and after acid hydrolysis, were made up to 250 μL with distilled water adjusted to pH 2.5 with trifluoroacetic acid and filtered through a 0.2-μm Anopore filter (Whatman, Maidstone, Kent, U.K.), prior to the analysis of 100-μL volumes ($^1/_{50}$ aliquot of total sample) by gradient elution reversed-phase HPLC.

High-Performance Liquid Chromatography. Samples were analyzed using a Shimadzu (Kyoto, Japan) LC-10A series automated liquid chromatograph comprising a SCL-10A system controller, two LC-10A pumps, a SIL-10A autoinjector with sample cooler, a CTO-10A column oven, and a SPD-10A UV−vis detector linked to a Reeve Analytical (Glasgow, U.K.) 2700 data handling system. Reversed-phase separations were carried out at 40 °C using a 150- × 3.0-mm i.d., 4-μm Genesis C18 cartridge column fitted with a 10- × 4.0-mm i.d., 4-μm C18 Genesis guard cartridge in an integrated holder (Jones Chromatography, Mid-Glamorgan, U.K.). The mobile phase was a 20-min, 20−40% gradient of acetonitrile in water adjusted to pH 2.5 with trifluoroacetic acid, eluted at a flow rate of 0.5 mL min^{-1}. Column eluent was monitored at 365 nm which provided a limit of detection of <5 ng (Crozier et al., 1997a). Linear 5−250-ng calibration curves were obtained for the flavonol aglycons morin, myricetin, quercetin, kaempfer-

Table 1. Details of Red Wines Analyzed for Free and Conjugated Flavonol Content[a]

wines	year	price (£)	label, region/country of origin; grapes; supplier
1, Valpolicella	1994	3.89	Gaetane Carron, Veneto, Italy; Corvina (70% max) with Rondinella and Molinara; Safeway
2, Valpolicella	1994	3.99	Fratelli Pasqua SpA, Verona, Italy; Corvina (70% max) with Rondinella and Molinara; Victoria Wine Cellars
3, Barolo	1993	7.99	Barolo, Cantina Terre del Barolo, Piedmont, S. Alba, Italy; 3-year-oak-aged, Nebbiolo; Victoria Wine Cellars
4, Chianti	1994	3.99	Piccini, Tuscany, Italy; Sangiovese (75% min) with Canaiolo, Trebbiano, and Malvasia; Victoria Wine
5, Chianti	1994	3.49	Il Pazazzo, Tuscany, Italy; Sangiovese (75% min) with Canaiolo, Trebbiano, and Malvasia; Oddbins
6, Chianti Classico	1991	6.99	Berardenga, Tuscany, Italy; Sangiovese (75% min) with Canaiolo, Trebbian, and Malvasia; Oddbins
7, Chianti Classico	1994	3.99	Piccini, Tuscany, Italy; Sangiovese (75% min) with Canaiolo, Trebbiano, and Malvasia; Safeway
8, Côtes du Rhône	1994	3.49	Enclave des Papes, Vaucluse, France; Grenache blended with Syrah and Cinsault; Oddbins
9, Côtes du Rhône	1992	7.69	Coudoulet de Beaucaster, Vaucluse, France; Grenache blended with Mourvèdre and Syrah; Oddbins
10, Châteauneuf du Pape	1993	9.99	Château de Vaudieu, Châteauneuf du Pape, France; Grenache blended with Syrah, Cinsault, and Cousant; Victoria Wine Cellars
11, L'Ardèche	1994	2.49	Les Vignerons Ardechois, Ruoms, France; Grenache (60%) and Syrah (40%); Safeway
12, Cabernet Sauvignon	1994	2.99	Lontue Region, Chile; Cabernet Sauvignon; Safeway
13, Cabernet Sauvignon	1995	3.99	Lontue Region, Chile; Cabernet Sauvignon; Safeway
14, Cabernet Sauvignon	1994	2.99	Lontue Region, Chile; Cabernet Sauvignon; Safeway
15, Cabernet Sauvignon	1992	4.99	Casillero del Diablo, Pirique Vineyard, Maipo Valley, Chile; Cabernet Sauvignon; Victoria Wine Cellars
16, Cabernet Sauvignon	1992	5.79	Viña Undurraga S.A., Santa Ana, Maipo Valley, Chile; Cabernet Sauvignon; Peckham & Rye
17, Cabernet Sauvignon	1992	4.99	Villa Montes, Discovery Wines, S.A., Curico Valley, Chile; oak-aged, Cabernet Sauvignon; Oddbins
18, Cabernet Sauvignon	1992	4.99	Villard Vineyards, Rancagua, Chile; oak-aged, Cabernet Sauvignon, harvested April 18, 1992; Safeway
19, Cabernet Sauvignon	1994	3.25	Vin de Pays D'Oc, Maisan Jean Saint Felix de Lodez, Herault, France; Cabernet Sauvignon; Oddbins
20, Cabernet Sauvignon	1994	4.79	Philip de Baudin, La Baume, Servian, France; Cabernet Sauvignon; Oddbins
21, Cabernet Sauvignon	1994	3.79	Barton & Guestier, Languedoc Region, France; Cabernet Sauvignon; Oddbins
22, Cabernet Sauvignon	1992	6.99	Franciscan Vineyards, Napa Valley, CA; Cabernet Sauvignon; Oddbins
23, Cabernet Sauvignon	ns	4.29	Ernest and Julio Gallo, California; Cabernet Sauvignon; Safeway
24, Cabernet Sauvignon	1993	4.79	Stoneybrook Vineyards, Redwood Valley, CA; Cabernet Sauvignon; Safeway
25, Cabernet Sauvignon	1994	4.49	Killawarra Vintage Wines, Nuriootpa, S.E. Australia; Cabernet Sauvignon; Oddbins
26, Cabernet Sauvignon	1992	8.49	Bin 407, Penfold Wines, Magill, S. Australia; Cabernet Sauvignon; Oddbins
27, Cabernet Sauvignon	1994	5.49	Montana, New Zealand; Cabernet Sauvignon from Malborough and Hawkes Bay; Oddbins
28, Cabernet Sauvignon	ns	2.50	Vinprom, Russe Region, Bulgaria; oak-aged, Cabernet Sauvignon; Sainsbury's
29, Cabernet Sauvignon	1989	3.89	Reserve Cabernet Sauvignon, Bulgarian Vinters, Vinpran, Rousse, Bulgaria; Cabernet Sauvignon; Victoria Wine Cellars
30, Cabernet Sauvignon	1990	3.99	Special Reserve Cabernet Sauvignon, Lovico Suhindol Winery, Suhindol, Bulgaria; Cabernet Sauvignon; Victoria Wine Cellars
31, Cabernet Sauvignon	1990	5.05	Gran Coronas, Spain; Cabernet Sauvignon (85%) and Tempranillo (15%); Allders, Glasgow Airport
32, Shiraz Cabernet Sauvignon	1994	3.99	Hardy's, S.E. Australia; Shiraz and Cabernet Sauvignon; Victoria Wine Cellars
33, Cabernet Sauvignon Merlot	1994	2.99	Rio Grande do Sol, Brazil; Cabernet Sauvignon and Merlot; Tesco Stores Ltd.
34, Graves	1993	8.99	Châteaux de L'Hospital, Girende, France; Cabernet Sauvignon, Cabernet Franc, and Merlot; Oddbins.
35, Bordeaux	1993	4.99	Calvet Réserve Bordeaux, J. Calvet & Co., Bordeaux Gironde, France; 18-month oak-aged, Cabernet Sauvignon, Cabernet Franc, and Merlot; Victoria Wine Cellars
36, Merlot	1994	4.49	Viña Undurraga S.A., Santa Ana, Maipo Valley, Chile; Merlot; Peckham & Rye
37, Merlot	1994	4.49	Errazuriz, Maule Valley, Chile; Merlot; Victoria Wine Cellars
38, Merlot	1993	7.99	Cuvéé Alexandre, Casa Lapostolle, Rapel Valley, Chile; oak-aged, Merlot; Oddbins
39, Merlot	1994	3.19	L'Ardech, France; Merlot; Safeway
40, Merlot	1994	2.50	La Baume Merlot, France; Merlot; Sainsbury's
41, Merlot	1995	4.59	Sutter Home Winery, Napa Valley, CA; Merlot; Peckham & Rye
42, Merlot	1994	4.99	Glen Ellen Winery and Vineyards, Sonoma, CA; Merlot; Safeway
43, Merlot	1993	7.99	Palacio de la Vega, Inversiones Arnotegul, Dicastillo, Navarra, Spain; Merlot; Oddbins
44, Merlot Pinot Noir	ns	2.99	Rouse Region, Bulgaria; Merlot and Pinot Noir; Safeway
45, Pinot Noir	1995	6.49	Special Reserve, Viña Cono Sur, Chimbarongo, Rapel, Chile; Pinot Noir; Oddbins
46, Pinot Noir	1995	4.39	Viña Tocornal, Cono Sur, Chimbarongo, Rapel, Chile; Pinot Noir; Victoria Wine Cellars
47, Pinot Noir	1993	6.99	Bourgogne du Chapitre, Jaffelin, Caves du Chapitre de Beaune, France; Pinot Noir; Oddbins
48, Pinot Noir	1992	6.99	Bourgogne, Hautes-Cotes de Beaune, Cote d'Or, France; Pinot Noir; Oddbins
49, Pinot Noir	1994	7.49	Pinot Noir Jadot, Louis Jadet, Négaciant-Eleveura, Beaune, France; Pinot Noir; Victoria Wine Cellars
50, Pinot Noir	1993	6.99	Franciscan Vineyards, Napa Valley, CA; Pinot Noir; Oddbins
51, Pinot Noir	ns	4.99	Havenscourt Vineyards, Healdsburg, CA; Pinot Noir; Oddbins
52, Pinot Noir	1994	8.99	Firesteed Cellars, Rickreall, OR; Pinot Noir; Peckham & Rye

Table 1 (Continued)

wines	year	price (£)	label, region/country of origin; grapes; supplier
53, Pinot Noir	1990	3.29	Dealul Region, Romania; Pinot Noir; Safeway
54, Beaujolais	1995	4.69	Olivier Ravier, Belleville, France; Gamay; Oddbins
55, Beaujolais	1995	3.49	M. Deschamps, Carnay-lès-Mâcon, France; Gamay; Safeway
56, Young Vatted Tempranillo	1995	2.49	Vinícola de Castilla, S.A., Manzanares, La Mancha, Spain; Tempranillo; Safeway
57, Rioja	ns	2.50	Denominacion de Origen Calificada, Spain; Tempranillo blended with Garnacho, Graziano, and Mazuelo; Sainsbury's
58, Tempranillo	1994	4.79	Bodegas Berberana, Cenicero, Rioja Alta, Spain; Tempranillo; Safeway
59, Minervois	ns	2.49	Minerve, France; Carignan (60% max) blended with Syrah, Cinsault, Mourvèdre, and Lladonner; Peckham & Rye
60, Minervois	1994	3.99	Domaine des Lauziers, Minerve, France; Carignan (60% max) with Syrah, Cinsault, Mourvèdre, and Lladonner; Safeway
61, Corbieres	ns	2.50	Corbières, France; Carignan (75% max) blended with Syrah, Grenache, and Mourvèdre; Sainsbury's
62, Côtes du Roussillon Villages	1995	3.69	Les Vignerons Catalans, Perpignon, France; Carignan (70% max) blended with Syrah and Mourvèdre (10% min) and other varieties; Safeway
63, Dorgan	ns	2.99	Aude Region, France; Carignan; Tesco Stores Ltd.
64, Doumaine Sapt-Inour	ns	2.99	N.E. Morocco; Carignan blended with Syrah and Cabernet Sauvignon; Safeway
65, Kékfrankos	1993	2.99	Great Plain, Kiskunfélegyháza Region, Hungary; Kékfrankos; Safeway

[a] ns, year not stated.

ol, and isorhamnetin. With the sample size analyzed, this facilitated a limit of detection of ca. 0.8 mg L^{-1} of wine.

In selected instances, putative flavonol HPLC peaks were collected after passing through the UV−vis detector and examined in a Unicam SP8-500 spectrophotometer (Pye Unicam, York, U.K.) to obtain 250−450-nm absorbance spectra for comparison with those of reference compounds. Further confirmation of identification was obtained by cochromatography of samples with standards. The acetonitrile-based gradient reversed-phase HPLC system readily resolves a range of flavonol and flavone standards except for luteolin and quercetin, which have very similar retention properties (Crozier et al., 1997a). Preliminary screening using an isocratic mobile phase of 50% methanol in water adjusted to pH 2.5 with trifluoroacetic acid, which provides a baseline separation of quercetin and luteolin (Crozier et al., 1997a), established that the wines contained quercetin, but not luteolin, in detectable quantities.

Estimates of Free and Conjugated Flavonol Levels. Free flavonols were detected in the unhydrolyzed samples, while the total flavonol content was determined after acid hydrolytic cleavage of the flavonol conjugates which released the aglycons. The conjugated flavonol level was, thus, estimated by subtracting the amount of flavonol found in the unhydrolyzed sample from the quantity present in the acid-hydrolyzed extract. With each analysis, the flavonol content of the hydrolyzed sample was corrected for sample handling/hydrolysis losses on the basis of the recovery of the morin internal standard, which typically was >90%. Triplicate samples from each bottle of wine were analyzed before and after acid hydrolysis.

Reference Compounds. Apigenin, kaempferol, morin, myricetin, quercetin, quercitrin (quercetin 3-L-rhamnoside), and rutin (quercetin 3β-D-rhamnoside) were purchased from Sigma Chemicals (Poole, Dorset, U.K.). Isorhamnetin, luteolin, and quercetin 3-glucoside were obtained from Apin Chemicals (Abingdon, Oxford, U.K.).

RESULTS AND DISCUSSION

Information on the 65 red wines analyzed in this study, their source, supplier, the grapes used, and the price are presented in Table 1, and their flavonol content, as determined by gradient reversed-phase HPLC, is listed in Table 2. The red wines contained myricetin and quercetin in both free and conjugated forms. Addition of these figures provides the total flavonol content of each wine for which a quercetin: myricetin ratio is also calculated. Typical HPLC traces obtained with the analysis of pre- and posthydrolyzed samples of wine 46, a Chilean Pinot Noir from the Rapel Valley, are illustrated in Figure 1.

There were very large variations in the flavonol content of the individual red wines, and there appear to be several interacting factors which may explain some of these differences. Quercetin glycosides accumulate in the skins of red grapes (Price et al., 1995), and the data in Table 2 indicate that wines containing higher concentrations of flavonols are, in the main, derived from “thick-skinned” grapes, such as Cabernet Sauvignon, which are characterized by a high skin:volume ratio, rather than “thinner-skinned” varieties such as Grenache with a low skin:volume ratio. There is also a trend toward higher flavonol levels in wines made from grapes grown in warmer sunnier climates, such as Chile, rather than cooler regions, such as northern Italy or northern France. This is in keeping with a report that Pinot Noir wines made from sun-exposed grape clusters contain over 7 times more quercetin glycosides than wines made from shaded berries (Price et al., 1995).

The time at which grapes are picked may also have a bearing on flavonol content in that there is an association between high-flavonol-containing wines and the use of very ripe grapes. This means that low flavonol levels are frequently a feature of wines from regions where a cool, damp climate dictates that grapes are often picked as soon as they reach a certain sugar level in order to minimize the risk of rain damaging the crop. In countries such as Chile, there is more control and the grapes are allowed to ripen to a much greater extent, and this appears to be associated with an increased accumulation of flavonols.

The production of high-flavonol wines is linked not only with the use of very ripe, thick-skinned grapes but also with the application of modern methods of vinification. This indicates that efficient extraction of flavonols requires proper, frequent pump over of the cap of skins, as well as the addition of pressings from the fermented skins. With these general observations in mind, consideration will now be given to levels of myricetin and quercetin in the individual wines listed in Table 2.

Italian Wines (Wines 1−7). Valpolicella (entries 1, 2) is from a cooler area in northern Italy using high-yielding, fairly thin-skinned Corvina, Rondinella, and Molinara grapes (see Table 1). The vines are trained on a pergola system which does not always enable full sunlight to reach the grapes. This could explain the low flavonol levels of <10 mg L^{-1}. Barolo (entry 3), which

Table 2. Free and Conjugated Myricetin and Quercetin Content of Different Types of Red Wines[a]

wines	myricetin free	myricetin conjugated	quercetin free	quercetin conjugated	total flavonol content	Q:M ratio	free flavonols (%)
1, Valpolicella (1994), Italy	0.5 ± 0.1	0.9 ± 0.2	0.3 ± 0.1	5.5 ± 0.4	7.2 ± 0.5	4.1	11
2, Valpolicella (1994), Italy	0.2 ± 0.1	1.2 ± 0.2	nd	4.6 ± 0.4	6.0 ± 0.7	3.3	3
3, Barolo (1993), Italy	0.3 ± 0.1	1.1 ± 0.2	1.5 ± 0.1	2.6 ± 0.4	5.5 ± 0.5	2.9	33
4, Chianti (1994), Italy	1.5 ± 0.1	0.9 ± 0.3	7.5 ± 0.1	5.8 ± 0.5	15.7 ± 0.7	5.5	57
5, Chianti (1994), Italy	0.3 ± 0.0	1.2 ± 0.2	7.0 ± 0.1	5.6 ± 0.3	14.1 ± 0.2	8.4	52
6, Chianti Classico (1991), Italy	2.1 ± 0.1	1.9 ± 0.1	13.2 ± 0.1	10.3 ± 0.3	27.5 ± 0.3	5.8	56
7, Chianti Classico (1994), Italy	1.8 ± 0.2	1.9 ± 0.2	9.2 ± 0.5	8.0 ± 0.9	20.9 ± 0.6	4.6	53
8, Côtes du Rhône (1994), France	1.3 ± 0.0	1.2 ± 0.1	1.5 ± 0.1	4.3 ± 0.1	8.3 ± 0.1	2.3	34
9, Côtes du Rhône (1992), France	1.9 ± 0.1	0.6 ± 0.1	2.6 ± 0.2	1.9 ± 0.3	7.0 ± 0.1	1.8	64
10, Châteauneuf du Pape (1993), France	2.0 ± 0.1	0.6 ± 0.3	0.8 ± 0.1	2.2 ± 0.4	5.6 ± 0.8	1.2	50
11, L'Ardèche (1994), France	1.5 ± 0.1	3.5 ± 0.2	nd	2.7 ± 0.1	7.7 ± 0.3	0.7	19
12, Cabernet Sauvignon (1994), Chile	1.8 ± 0.1	10.8 ± 2.3	7.2 ± 0.3	21.8 ± 1.2	41.6 ± 2.0	2.3	22
13, Cabernet Sauvignon (1995), Chile	4.2 ± 0.4	14.0 ± 0.1	7.3 ± 0.4	15.8 ± 0.4	41.3 ± 1.1	1.3	28
14, Cabernet Sauvignon (1994), Chile	1.4 ± 0.1	18.4 ± 0.7	2.8 ± 0.1	14.2 ± 1.1	36.8 ± 1.9	0.9	11
15, Cabernet Sauvignon (1992), Chile	8.4 ± 0.3	11.0 ± 0.6	7.0 ± 0.3	4.3 ± 0.2	30.7 ± 0.5	0.6	50
16, Cabernet Sauvignon (1992), Chile	3.5 ± 0.1	9.9 ± 0.3	1.3 ± 0.1	5.9 ± 0.3	20.6 ± 0.6	0.5	23
17, Cabernet Sauvignon (1992), Chile	2.0 ± 0.2	9.4 ± 0.3	1.1 ± 0.1	4.3 ± 0.6	16.8 ± 0.9	0.5	18
18, Cabernet Sauvignon (1992), Chile	4.6 ± 0.1	6.8 ± 0.7	0.9 ± 0.1	3.7 ± 0.3	16.0 ± 0.9	0.4	34
19, Cabernet Sauvignon (1994), France	6.7 ± 0.1	7.5 ± 0.5	4.6 ± 0.1	5.6 ± 0.2	24.4 ± 0.9	0.7	46
20, Cabernet Sauvignon (1994), France	4.6 ± 0.1	2.6 ± 0.5	3.7 ± 0.1	5.4 ± 0.4	16.3 ± 0.9	1.3	51
21, Cabernet Sauvignon (1994), France	3.2 ± 0.1	4.2 ± 0.4	2.6 ± 0.2	3.1 ± 0.3	13.1 ± 0.8	0.8	44
22, Cabernet Sauvignon (1992), California	4.9 ± 0.1	10.8 ± 0.3	7.1 ± 0.3	10.8 ± 1.3	33.6 ± 1.6	1.1	36
23, Cabernet Sauvignon (ns), California	1.9 ± 0.1	4.7 ± 0.3	3.8 ± 0.1	10.3 ± 0.4	20.7 ± 0.5	2.1	27
24, Cabernet Sauvignon (1993), California	3.2 ± 0.1	3.2 ± 0.3	1.9 ± 0.1	5.1 ± 0.5	13.4 ± 0.7	1.1	38
25, Cabernet Sauvignon (1994), Australia	6.2 ± 0.2	5.4 + 0.3	5.5 ± 0.1	5.0 ± 0.2	22.1 ± 0.5	0.9	53
26, Cabernet Sauvignon (1992), Australia	6.7 ± 0.3	4.8 + 0.2	3.0 ± 0.2	3.3 ± 0.1	17.8 ± 0.6	0.5	55
27, Cabernet Sauvignon (1994), New Zealand	2.4 ± 0.1	4.4 ± 0.2	1.9 ± 0.1	8.1 ± 0.4	16.8 ± 0.6	1.5	26
28, Cabernet Sauvignon (ns), Bulgaria	1.1 ± 0.1	2.5 ± 0.2	nd	1.6 ± 0.1	5.2 ± 0.3	0.4	21
29, Cabernet Sauvignon (1989), Bulgaria	1.2 ± 0.1	2.0 + 0.1	0.4 ± 0.1	2.0 ± 0.1	5.6 ± 0.2	0.7	29
30, Cabernet Sauvignon (1990), Bulgaria	2.0 ± 0.1	1.1 + 0.2	1.2 ± 0.1	1.2 ± 0.1	5.5 ± 0.3	0.8	58
31, Cabernet Sauvignon, (1990), Spain	2.8 ± 0.0	1.6 ± 0.1	3.0 ± 0.0	3.3 ± 0.1	10.7 ± 0.1	1.4	54
32, Cabernet Sauvignon/Shiraz (1994), Australia	2.4 ± 0.1	0.3 ± 0.1	9.7 ± 1.2	5.6 ± 0.3	18.0 ± 0.9	5.7	67
33, Cabernet Sauvignon/Merlot (1994), Brazil	1.1 ± 0.0	3.4 ± 0.4	1.2 ± 0.0	8.1 ± 0.1	13.8 ± 0.4	2.1	17
34, Graves (1993), France	3.3 ± 0.1	10.2 ± 0.2	1.6 ± 0.1	6.1 ± 0.6	21.2 ± 0.8	0.6	23
35, Bordeaux (1993), France	1.1 ± 0.1	3.9 ± 0.1	1.0 ± 0.1	2.3 ± 0.2	8.3 ± 0.2	0.7	25
36, Merlot (1994), Chile	2.9 ± 0.4	8.9 ± 0.3	9.6 ± 0.3	14.4 ± 0.5	35.8 ± 0.9	1.9	35
37, Merlot (1993), Chile	5.0 ± 0.1	11.3 ± 0.4	4.1 ± 0.0	6.3 ± 0.2	26.7 ± 0.7	0.6	34
38, Merlot (1994), Chile	2.5 ± 0.2	12.6 ± 1.0	1.9 ± 0.1	8.4 ± 0.6	25.4 ± 1.3	0.7	17
39, Merlot (1994), France	4.0 + 0.1	2.4 + 0.5	9.5 + 0.2	7.7 + 0.3	23.6 ± 0.6	2.7	57
40, Merlot (1994), France	4.0 ± 0.2	3.9 ± 0.4	6.0 ± 0.1	5.2 ± 0.7	19.1 ± 1.2	1.4	52
41, Merlot (1995), California	0.9 ± 0.0	11.1 ± 0.3	4.0 ± 0.1	13.2 ± 0.8	29.2 ± 1.1	2.3	17
42, Merlot (1994), California	1.5 ± 0.1	3.4 ± 0.1	2.3 ± 0.1	7.4 ± 0.3	14.6 ± 0.3	2.0	26
43, Merlot (1993), Spain	5.1 ± 0.1	10.3 ± 0.2	6.8 ± 0.1	8.8 ± 0.6	31.0 ± 0.8	1.0	38
44, Merlot Pinot/Noir (ns), Bulgaria	1.3 ± 0.1	0.9 ± 0.2	0.6 ± 0.1	2.2 ± 0.4	5.0 ± 0.4	1.3	38
45, Pinot Noir (1995), Chile	4.2 ± 0.5	5.5 ± 0.9	6.5 ± 0.4	13.2 ± 1.8	29.4 ± 3.0	2.0	36
46, Pinot Noir (1995), Chile	3.8 ± 0.4	4.8 ± 0.3	5.9 ± 0.2	10.6 ± 0.2	25.1 ± 0.5	1.9	39
47, Pinot Noir (1993), France	2.5 ± 0.1	1.6 ± 0.4	2.5 ± 0.2	2.6 ± 0.2	9.2 ± 0.6	1.2	54
48, Pinot Noir (1992), France	1.3 ± 0.2	0.8 ± 0.5	2.6 ± 0.2	3.8 ± 0.5	8.5 ± 0.3	3.0	46
49, Pinot Noir (1994), France	1.3 ± 0.0	0.8 ± 0.1	0.9 ± 0.2	2.8 ± 0.1	5.8 ± 0.2	1.8	40
50, Pinot Noir (1993), California	2.8 ± 0.1	2.1 ± 0.3	3.8 ± 0.1	5.3 ± 1.0	14.0 ± 0.9	1.9	47
51, Pinot Noir (ns), California	3.0 ± 0.1	1.6 ± 0.9	2.8 ± 0.1	4.5 ± 1.7	11.9 ± 2.5	1.6	49
52, Pinot Noir (1994), Oregon	1.8 ± 0.2	1.9 ± 0.3	1.9 ± 0.0	3.0 ± 0.2	8.6 ± 0.3	1.3	43
53, Pinot Noir (1990), Romania	1.2 ± 0.1	0.7 ± 0.1	3.0 ± 0.1	2.9 ± 0.2	7.8 ± 0.3	3.1	54
54, Beaujolais (1995), France	0.8 ± 0.1	1.3 ± 0.1	1.1 ± 0.1	1.8 ± 0.2	5.0 ± 0.2	1.4	38
55, Beaujolais (1995), France	0.9 ± 0.1	1.8 ± 0.1	nd	2.0 ± 0.1	4.7 ± 0.1	0.7	19
56, Young Vatted Tempranillo (1995), Spain	1.7 ± 0.1	13.6 ± 0.3	1.9 ± 0.1	5.4 ± 0.1	22.6 ± 0.4	0.5	16
57, Rioja (ns), Spain	2.4 ± 0.1	6.3 ± 1.6	nd	2.2 ± 0.0	10.9 ± 0.4	0.3	22
58, Tempranillo (1994), Spain	2.8 ± 0.3	3.4 ± 0.5	0.1 ± 0.0	3.0 ± 0.3	9.3 ± 1.0	0.5	31
59, Minervois (ns), France	5.4 ± 0.2	1.7 ± 0.2	15.8 ± 0.4	13.1 ± 0.5	36.0 ± 1.2	4.0	59
60, Minervois (1994), France	6.3 ± 0.2	7.9 ± 1.0	5.8 ± 0.2	5.0 ± 0.1	25.0 ± 1.0	0.8	48
61, Corbieres (ns), France	5.6 ± 0.1	3.7 ± 0.4	5.2 ± 0.1	2.6 ± 0.6	17.1 ± 0.9	0.8	63
62, Côtes du Roussillon Villages (1995), France	5.8 ± 0.1	4.8 ± 0.2	1.2 ± 0.2	3.6 ± 0.3	15.4 ± 0.4	0.5	45
63, Dorgan (ns), France	3.3 ± 0.1	4.2 ± 0.2	0.6 ± 0.2	3.5 ± 0.4	11.6 ± 0.7	0.5	34
64, Doumaine Sapt-Inour (ns), Morocco	1.4 ± 0.1	4.6 ± 0.2	11.4 ± 0.3	16.9 ± 0.4	34.3 ± 0.2	4.7	37
65, Kékfrankos (1993), Hungary	0.9 ± 0.1	0.1 ± 0.1	1.9 ± 0.1	1.7 ± 0.2	4.6 ± 0.3	3.6	60

[a] Data are expressed as mg L^{-1} ± SE ($n = 3$). nd, not detected; ns, year not stated; Q:M ratio, quercetin:myricetin ratio; free flavonols (%), − level of free flavonols expressed as a percentage of total flavonol content.

is also produced in a fairly cool climate in northern Italy, similarly has a very low flavonol content, 5.5 mg L^{-1}. Although the Nebbiolo grapes are relatively thick-skinned, the wine was probably made with a fairly light extraction, which has been widely adopted to get away from some of the rather tough tannic wines that used to be characteristic of this region. Chianti is produced in the Tuscany region, principally from Sangiovese grapes which have a medium skin. Despite being coolish, Tuscany is further south than Barolo, and these factors probably account for the higher flavonol levels of ca. 15 mg L^{-1} in wines 4 and 5. Chianti Classico is

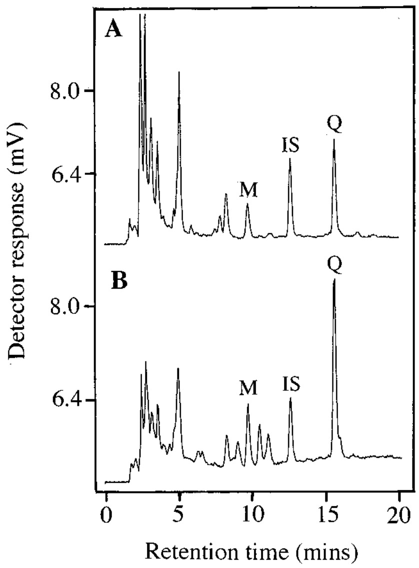


Figure 1. Gradient, reversed-phase HPLC analysis of free and conjugated myricetin and quercetin in wine 46, a 1994 Chilean Pinot Noir. Column: 150- × 3.0-mm i.d., 4-μm Genesis C18 cartridge column with a 10- × 4.0-mm i.d., 4-μm C18 Genesis guard cartridge at 40 °C. Mobile phase: 20-min gradient of 20−40% acetonitrile in water adjusted to pH 2.5 with TFA. Flow rate: 0.5 mL min^{-1}. Samples: extract aliquot (A) before acid hydrolysis at 90 °C in 1.2 M HCl for 2 h and (B) after acid hydrolysis. Detector: absorbance monitor operating at 365 nm. IS, morin internal standard; M, myricetin; Q, quercetin.

made from lower-yielding grapes which are grown in a sunnier microclimate and ripen better, a factor which may explain the increase in the flavonol content of wines 6 and 7 to 27.5 and 20.9 mg L^{-1}, respectively.

Interestingly the seven Italian wines had a higher quercetin:myricetin ratio than the many of the other wines that were investigated (Table 2).

Côtes du Rhône Type Wines (Wines 8−11). Côtes du Rhône is made principally from Grenache grapes, a thin-skinned, light-colored variety. The grapes are also picked a little early in the season, and these two factors probably account for the low flavonol content of wines 8 and 9. Châteauneuf du Pape has a lower percentage of Grenache and a higher percentage of thicker-skinned grapes such as Syrah, which are grown in an area where the terroir (the effects of the soil and local microclimate) allows the grapes to ripen more than in the Côtes du Rhône area. It is therefore surprising that wine 10 has a flavonol content of only 5.6 mg L^{-1}. This is likely to be a consequence of limited extraction of the skins as this wine is light in style for a Châteauneuf du Pape. The Ardeche has similar ripening conditions to the Côtes du Rhône, and similar varieties of grapes are used; hence, the relatively low flavonol content of wine 11, 7.7 mg L^{-1}, is not unexpected.

Cabernet Sauvignon (Wines 12−35). Cabernet Sauvignon grapes have a high skin:volume ratio which appears to be associated with the production of flavonol-rich wines. In most regions of Chile the grapes ripen well, with ample sunlight and irrigation to provide excellent growing conditions. Chilean wines sold in the U.K. are usually from good wineries which, in recent years, have utilized modern vinification techniques. This is reflected in the ca. 40 mg L^{-1} flavonol content of wines 12−14, which were 1994 and 1995 vintages of Cabernet Sauvignon from the same Lontué vineyard in the Curicó Valley. The lower flavonol content of some older Chilean Cabernet Sauvignons from vineyards in the Curicó and Maipo Valleys (entries 16−18) may be indicative of the use of more traditional methods of wine making.

French Cabernet Sauvignon wines (entries 19−21) are made from grapes grown in the south of France that do not usually achieve the same degree of ripeness as those from Chile. In addition, irrigation of vines is not permitted in France, and the soil may not be as nutritious as in Chile. All of these factors may have a bearing on the flavonol levels in French Cabernet Sauvignon which are generally lower than in the Chilean wines. Wines 19 and 20 may not have had as much extraction as the Chilean wines, but wine 20 is made with modern extraction techniques yet still contains only 16.3 mg of total flavonol L^{-1}. This relatively low concentration is, therefore, likely to be due to the flavonol content of the grapes themselves.

Both Californian and Australian vineyards have a lot of sun to ripen the grapes, irrigation is used to achieve excellent growing conditions, and in general, modern methods of vinification are used. This is reflected in the total flavonol content of 33.6 mg L^{-1} found in wine 22 which is from the Franciscan Vineyards in the Napa Valley. The levels of flavonols in other Californian and Australian Cabernet Sauvignon wines (entries 23−26) are, however, unexpectedly low compared to their Chilean counterparts. New Zealand is a relatively cool area, but the better vineyards are expert at leaving the grapes to ripen for as long as possible and in training vines so that berry clusters gain maximum exposure to the sun. This could explain why wine 27 has a total flavonol content of 16.8 mg L^{-1}, which is somewhat higher than might be expected in the cooler New Zealand climate.

The 1989 and 1990 Cabernet Sauvignon wines from Bulgaria (entries 28−30) are extremely low in flavonols, barely reaching 5 mg L^{-1}. In most years Bulgaria has hot and sunny ripening conditions which are conducive to the accumulation of high flavonol levels in berries (Price et al., 1995). The extremely low myricetin and quercetin content of these wines is therefore likely to be due to the grapes being picked too early, the canopy of leaves preventing the sun from shining directly on the grapes, and the use of wine-making procedures that include poor extraction of the skins, removing the skins too early, and little addition back of pressings. Improved methods of wine production are being introduced in Bulgaria so it would be of interest to analyze later vintages from modernized wineries. The sole Spanish Cabernet Sauvignon (entry 31) that was analyzed had a total flavonol content of 10.7 mg L^{-1}. Although Spain is sunny, sometimes wine making is not as advanced as in Chile, and this relatively low level of flavonols is most likely due to a lack of irrigation and a relatively poor terroir coupled with inefficient extraction of the grapes.

The blended Cabernet Sauvignon wines (entries 32−35) contained variable concentrations of flavonols. The highest quantity of total flavonols, 21.2 mg L^{-1}, was in wine 34, a Graves from Châteaux de L'Hospital. This wine is from a well-cared for vineyard, in a good ripening region, that uses modern methods of vinification. The low flavonoid content of the Calvet Réserve Bordeaux (entry 35) is probably due to the fact that the grapes in many Bordeaux vineyards struggle to ripen.

Merlot (Wines 36−44). Among the Merlot wines, as with Cabernet Sauvignon, the Chilean wines from the Maipo and Maule Valleys (entries 36−38) again contain high concentrations of flavonols which varied less than the levels in Merlot from other countries (entries 39−44). In this instance, the single Spanish wine that was analyzed (entry 43) scored well, containing 31 mg of total flavonol L^{-1}. The Bulgarian Merlot/Pinot Noir contained only 5.0 mg L^{-1}, which once again is probably indicative of the use of traditional wine-making techniques in this region.

Pinot Noir (Wines 45−53). Pinot Noir is a thin-skinned grape which, especially when grown in the cooler French climate, gives rise to high-quality, but low-flavonol-containing wines (entries 47−49). The flavonol contents of Pinot Noir wines from California and Romania (entries 50−53) were also low, ranging from 7.8 to 14.0 mg L^{-1}. This was well below the concentrations detected in the two Chilean wines from vineyards in the Rapel Valley (entries 45, 46), which were a remarkable 29.4 and 25.1 mg L^{-1}. Pinot Noir grapes contain conjugated rather than free quercetin (Price et al., 1995). In contrast, wines 45 and 46, along with many of the other wines that were analyzed (see Table 2), contained significant quantities of free quercetin and free myricetin. Presumably this is due to enzymatic hydrolysis of flavonol conjugates during vinification and/or maturation of the wines

Others (Wines 54−65). The low flavonol content of the two samples of Beaujolais (entries 54, 55), at 4.7 and 5.0 mg L^{-1}, is probably a consequence of the mild extraction of Gamay grapes harvested when they are not quite at full ripeness. Two Spanish wines produced from Tempranillo grapes (entries 57, 58) contained relatively low levels of flavonols at 10.9 and 9.3 mg L^{-1}. In contrast, wine 56, a Young Vatted Tempranillo, had a total flavonol content of 22.6 mg L^{-1}, which suggests that when Tempranillo berries, which are fairly thin-skinned, are left on the vine to ripen fully, there is a marked increase in the accumulation of myricetin and quercetin. The Minervois wines 60 and 61 from southern France are produced from well-ripened Carignan grapes blended with other varieties including thick-skinned Syrah berries, and this is reflected in flavonoid contents of 36.0 and 25.0 mg L^{-1}. Although wines 62 and 63 are also based on Carignan grapes, they are from regions where berries do not achieve full ripeness, and this is reflected in the lower concentration of flavonols, 15.4 and 11.6 mg L^{-1}, respectively, in these wines. The Moroccan wine (entry 64) contained a relatively high 34.3 mg of total flavonols L^{-1}. It is produced from fully ripe Carignan grapes blended with Syrah and Cabernet Sauvignon, which are grown in a hot, sunny region in conditions that are conducive to the accumulation of flavonols. In contrast, the Hungarian Kékfrankos wine (entry 64) which contains only 4.6 mg L^{-1} is produced from thin-skinned grapes that are grown in a much cooler climate.

Wines aged in oak contain higher amounts of derivatives of hydroxybenzoic acid, cinnamaldehyde, and benzaldehyde, but there are no reports of flavonols being influenced significantly (Singleton, 1995; Soleas et al., 1997). Three of the wines (entries 3, 35, 38) analyzed in the present study were aged in oak, but this is too few samples to draw any inferences about possible effects of the process on flavonol levels.

The data presented in this paper demonstrate that there is considerable variation in the total flavonol content of red wines which ranges from 4.6 to 41.6 mg L^{-1} (Table 2). In a previous study, the combined levels of myricetin and quercetin in Californian red wines were found to vary from 6.8 to 25.7 mg L^{-1} (Frankel et al., 1995). Red wines contain sizable amounts of free myricetin and quercetin, typically 20−50% of the total flavonol content (Table 2). This is in marked contrast to grapes (Stewart and Crozier, unpublished data), other fruits, and vegetables, as well as beverages such as tea, in which conjugated flavonols dominate and aglycons are present, at best, in only trace amounts (Herrmann, 1988; Hertog et al., 1992a, 1993b; Crozier et al., 1997b). Typically, free flavonols are much more active than their conjugated derivatives in both antioxidant and anti-platelet aggregation assays [see Frankel et al. (1995), Vinson et al. (1995)], and this may be a contributory factor in explaining the protective effects of red wine against cardiovascular disease. Although preliminary reports are beginning to appear in the literature on the flavonol content of plasma and urine (Hollman et al., 1995, 1996), there is still a dearth of detailed information on the effectiveness with which different conjugated flavonols, and their aglycons, are absorbed and the extent to which they are subsequently metabolized. Ultimately, the degree of protection afforded by red wines, as well as fruits, vegetables, and other beverages, will be determined by (i) the efficiency with which the different types of potential antioxidants are absorbed, (ii) their form and residence time in the blood and relevant tissues, and (iii) the resultant in vivo biological activity.

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