Content of the Flavonols Myricetin, Quercetin, and Kaempferol in Finnish Berry Wines

Helena Vuorinen,*[,†,§] Kaisu Määttä,[†,§] and Riitta Törrönen[†,‡]

Departments of Physiology and Clinical Nutrition, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland, and Finnish Research and Information Center for Fruit Wines, Hietapohjantie 926, FIN-73460 Muuruvesi, Finland

The amounts of myricetin, quercetin, and kaempferol were analyzed in 16 red and 2 white berry and grape wines after acid hydrolysis using an RP-HPLC method with diode array detection. The red berry wines analyzed were made mainly from black currant, crowberry, and bog whortleberry, i.e., berries rich in flavonols. The red grape wines were made mainly from Cabernet Sauvignon or Merlot grapes in several countries. The white wines studied were gooseberry and white currant wines and Chardonnay and Riesling wines. The amount of myricetin ranged from 3.8 to 22.6 mg L^{-1} in red berry wines and from 0 to 14.6 mg L^{-1} in red grape wines. The amount of quercetin was from 2.2 to 24.3 mg L^{-1} in red berry wines and from <1.2 to 19.4 mg L^{-1} in red grape wines. Low levels of kaempferol were found in all red berry wines and in 9 red grape wines. The total concentration of these flavonols was from 6 to 46 mg L^{-1} (mean 20 mg L^{-1}) in red berry wines and from 4 to 31 mg L^{-1} (mean 15 mg L^{-1}) in red grape wines. Small amounts of quercetin were found in white currant and gooseberry wines, whereas no flavonols were detected in white grape wines. These results demonstrate that the contents of flavonols in red berry wines are comparable to those in red grape wines.

Keywords: *Flavonol; myricetin; quercetin; kaempferol; berry wine*

INTRODUCTION

Epidemiological evidence from many studies suggests that moderate wine consumption is associated with a reduced risk of coronary heart disease (CHD) (reviewed by Constant, 1997; Soleas et al., 1997a). In France, CHD mortality is lower than in other industrialized countries, despite the high incidence of risk factors, such as high intake of saturated fats. Regular consumption of red wine has been proposed to be the most likely cause for this phenomenon known as the French paradox (Renaud and de Lorgeril, 1992).

Some of the protective effects of wine may be attributed to ethanol, the second largest component of wine (Soleas et al., 1997a). However, the superior benefits of wine, especially of red wine, over other alcoholic beverages cannot be explained solely by its content of ethanol. Red wine contains a wide range of polyphenolic constituents, including monomeric flavonoids and phenolic acids as well as polymeric tannins. Frankel et al. (1993) showed that the phenolic substances in red wine inhibit the oxidation of human low-density lipoprotein in vitro. Since then, the antioxidant activity of red wine polyphenolics has been demonstrated in many experimental systems. The antioxidant function as well as many other desirable biological activities of wine polyphenolics have been recently reviewed by Soleas et al. (1997a) and by Lairon and Amiot (1999).

The flavonoid fraction of wine consists of flavonols, catechins (flavan-3-ols), and anthocyanins (Soleas et al., 1997a). During the past decade, flavonols have received much attention due to their plausible benefits on human health. The antioxidative, antiplatelet aggregation, anti-inflammatory, antimutagenic, anticarcinogenic, and antiviral activities of quercetin and other flavonols have been demonstrated in vitro (Formica and Regelson, 1995; Hertog and Hollman, 1996; Soleas et al., 1997a). Epidemiological studies indicate that high consumption of flavonol-rich foods and beverages may be protective against CHD (Hertog et al., 1993a, 1995, 1997; Knekt et al., 1996), stroke (Keli et al., 1996), lung cancer (Knekt et al., 1997), and stomach cancer (Garcia-Closas et al., 1999).

Long-term health benefits associated with flavonols have increased the interest in the content of these flavonoids in various foods and beverages. Recent studies (Häkkinen and Auriola, 1998; Häkkinen et al., 1998, 1999a,b) have shown that many berries are rich in flavonols. The total content of the flavonols quercetin, myricetin, and kaempferol is higher in cranberry, bog whortleberry, lingonberry, crowberry, and black currant than in the commonly consumed fruits or vegetables, with the exceptions of onion and kale (Häkkinen et al., 1999b).

In Finland, berries such as black, red, and white currants, gooseberry, strawberry, red raspberry, bilberry, bog whortleberry, and crowberry are used for the production of wines. In contrast to grape wines, the phenolic composition of berry wines is poorly known. By using the Folin−Ciocalteu procedure, Heinonen et al. (1998) measured the total phenolic contents of 27 berry wines. Lehtonen et al. (1999) quantified several

* To whom correspondence should be addressed at Department of Physiology, University of Kuopio. Tel: + 358 17 163 103. Fax: +358 17 163 112. E-mail: Helena.Vuorinen@uku.fi.
† Department of Physiology, University of Kuopio.
‡ Department of Clinical Nutrition, University of Kuopio.
§ Finnish Research and Information Center for Fruit Wines.

10.1021/jf991388o CCC: $19.00
Published on Web 06/03/2000

Table 1. Flavonol Contents of Finnish Berry Wines (mg/L)

raw materials	myricetin[a]	quercetin[a]	kaempherol[a]	sum
red berry wines				
1 black currant	22.6 ± 0.6	11.9 ± 0.2	<1.2	34.5
2 black currant	14.6 ± 0.1	8.8 ± 0.0	<1.2	23.5
3 black currant	14.5 ± 0.2	8.7 ± 0.1	<1.2	23.1
4 black currant	11.2 ± 0.0	9.8 ± 0.0	2.1 ± 0.0	23.0
5 black currant	13.2 ± 0.1	8.3 ± 0.1	<1.2	21.5
6 black currant	7.3 ± 0.1	3.1 ± 0.0	<1.2	10.4
7 black currant, red currant	10.3 ± 0.3	6.7 ± 0.1	<1.2	17.0
8 black currant, strawberry	10.5 ± 0.4	11.1 ± 0.4	3.3 ± 0.1	24.9
9 black currant, raspberry, strawberry	14.8 ± 0.2	8.5 ± 0.1	<1.2	23.3
10 black currant, crowberry	13.0 ± 0.1	10.2 ± 0.0	<1.2	23.3
11 black currant, crowberry	6.5 ± 0.1	4.0 ± 0.1	<1.2	10.5
12 black currant, crowberry	5.7 ± 0.0	3.7 ± 0.0	<1.2	9.4
13 black currant, crowberry	4.8 ± 0.2	3.0 ± 0.2	<1.2	7.8
14 black currant, crowberry, rose hip	3.8 ± 0.1	2.2 ± 0.1	<1.2	6.0
15 crowberry	7.2 ± 0.1	7.4 ± 0.1	<1.2	14.6
16 bog whortleberry, strawberry, black currant, crowberry	22.1 ± 0.2	24.3 ± 0.5	<1.2	46.4
white berry wines				
17 white currant	nd	4.1 ± 0.1	nd	4.1
18 gooseberry	nd	<1.2	nd	0.0

[a] Mean of triplicate assays ± SEM; nd, not detectable.

phenolic compounds from 8 berry wines using high-performance liquid chromatography (HPLC). Berry wines have also been shown to possess antioxidant activity on the oxidation of methyl linoleate, wines made from black currant and crowberry or bilberry being slightly superior to red grape wines (Heinonen et al., 1998).

The aim of the present study was to investigate the amounts of the flavonols myricetin, quercetin, and kaempferol as aglycons in berry wines. The wines analyzed were made mainly from black currant, crowberry, and bog whortleberry, i.e., berries rich in flavonols (Häkkinen et al., 1999b). Grape wines made from Cabernet Sauvignon, Merlot, Chardonnay, or Riesling grapes in several countries as well as some other wines commonly consumed in Finland were analyzed as reference wines.

MATERIALS AND METHODS

Wines. The berry wines (Table 1) were from 9 different producers mainly from eastern Finland. Six red berry wines were made from black currant (*Ribes nigrum*) only. In addition to black currant, 8 red berry wines contained also either strawberry (*Fragaria x ananassa*), red raspberry (*Rubus idaeus*), red currant (*Ribes x pallidum*), rose hip (*Rosa* sp.), or wild crowberry (*Empetrum nigrum*). One red berry wine was made from crowberry only, and in one red berry wine the main component was wild bog whortleberry (*Vaccinum uliginosum*). White berry wines were made from white currant *(Ribes x pallidum*) or gooseberry (*Ribes uva-crispa*). In general, the process of producing berry wines follows the production of grape wines. However, berries contain high amounts of acids and relatively low amounts of sugars. Therefore, addition of water and sugar is necessary for getting berry juice to ferment for wine.

The Cabernet Sauvignon and Merlot wines were from Australia, Bulgaria, California, or Chile (Table 2). The other red wines were made from various grapes in Italy, France, or Spain. The two white wines were a Chardonnay wine from California and a Riesling wine from Germany.

Extraction and Hydrolysis Conditions. Flavonols were extracted and hydrolyzed to aglycons with a method modified from the procedures optimized for berries (Häkkinen and Auriola, 1998; Häkkinen et al., 1999b) or grape wines (Hertog et al., 1993b; McDonald et al., 1998; Justesen et al., 1998). Wine samples (15 mL) were refluxed for 1 h at 85 °C in 50% (v/v) methanol containing hydrochloric acid (0.5 M) and morin as an internal standard. The cooled samples were filtered through a 0.45-μm RC filter (SRI, Roth, Germany) prior to injection of 10 μL to HPLC. Triplicate samples (from one bottle) of each wine were prepared.

According to Häkkinen et al. (1998, 1999b) and Justesen et al. (1998), flavonol aglycons are sensitive during and after acid hydrolysis (1.2 or 0.9 M HCl) and the recoveries of added flavonols, especially that of myricetin, are low in some sample matrixes. In the present study, these problems were avoided by lowering the acid concentration. The concentration of 0.5 M was chosen after testing several concentrations between 0.3 and 1.2 M for the efficiency in breaking the glycosidic bonds versus the increase of the baseline (Figure 1). No losses of flavonols were measured in hydrolyzed wine samples during 13 h at room temperature, and the recoveries of added flavonols were 80−105%.

Direct Separation. All wines were also analyzed directly without extraction and hydrolysis. The wine samples were filtered as above and injected (10 μL) as such to HPLC.

High-Performance Liquid Chromatography. Samples were analyzed using a Hewlett-Packard (Waldbronn Analytical Division, Germany) instrument with a 1100 series quaternary pump, an autosampler, and a diode array detector linked to a HPChemStation data handling system. Reverse-phase separations were carried out using a LiChroCART column (125- × 3-mm i.d., 5-μm Purospher RP-18e; Merck, Darmstadt, Germany) with a LiChroCART guard column (4 × 4 mm). The flavonols were separated with gradient elution (Figure 1) using acetonitrile (A) and 1% formic acid (B) as follows:

$$0 \xrightarrow[0.4\ \text{mL/min}]{5-40\%\ \text{A}} 10 \xrightarrow[0.5\ \text{mL/min}]{40-70\%\ \text{A}} 20 \xrightarrow[0.5\ \text{mL/min}]{70-90\%\ \text{A}} 22 \xrightarrow[0.4\ \text{mL/min}]{90-5\%\ \text{A}} 25\ \text{min}$$

Flavonols were detected at 360 nm and identified according to the retention times and UV spectra of standards. Quantification was performed using the ratio of the UV responses of the internal standard (morin) and the identified compounds.

Analytical Quality Control. This method facilitated a limit of quantification of ca. 1 mg L^{-1}, and the limit of detection was 0.5 mg L^{-1}. Precision of the procedure was assessed for black currant wine 4. The coefficients of variation (CV) for the repeatability of the HPLC analysis ($n = 6$) were 3.9, 2.6, and 1.5% for myricetin, quercetin, and kaempferol, respectively. The CVs for the repeatability of the whole method ($n = 8$) were 7.1, 6.5, and 5.5% for myricetin, quercetin, and kaempferol, respectively. The recoveries of standard compounds added into black currant wine 5 were 92−105% for myricetin, 90−100% for quercetin, 80−88% for kaempferol, and 80−99% for morin.

Table 2. Flavonol Contents of Grape Wines (mg/L)

wine	country	myricetin[a]	quercetin[a]	kaempherol[a]	sum
Cabernet Sauvignon wines					
Sonoma County Cabernet Sauvignon, 1994	California	14.6 ± 0.2	10.0 ± 0.9	<1.2	24.6
St. George Cabernet Sauvignon, 1993	Australia	11.9 ± 0.0	10.2 ± 0.0	<1.2	22.1
Gallo Cabernet Sauvignon, 1997	California	9.5 ± 0.1	6.7 ± 0.1	<1.2	16.3
Gato Negro Cabernet Sauvignon	Chile	10.4 ± 0.2	4.4 ± 0.0	<1.2	14.8
Santa Carolina Cabernet Sauvignon, 1997	Chile	9.6 ± 0.1	3.0 ± 0.1	<1.2	12.6
Oriachovitza Cabernet Sauvignon Reserve, 1995	Bulgaria	4.6 ± 0.2	1.4 ± 0.3	nd	6.0
Sophia Cabernet Sauvignon, 1994	Bulgaria	3.5 ± 0.1	<1.2	<1.2	3.5
Merlot wines					
Brown Brothers Merlot, 1996	Australia	11.8 ± 0.3	19.4 ± 0.6	<1.2	31.2
Gato Negro Merlot	Chile	9.6 ± 0.1	7.1 ± 0.1	nd	16.8
Gallo Turning Leaf Merlot, 1996	California	5.9 + 0.1	9.6 ± 0.1	nd	15.5
Sophia Merlot, 1995	Bulgaria	nd	5.4 ± 0.3	nd	5.4
other red wines					
Sangre de Toro (grapes: Garnacha, Gariñena)	Spain	11.0 ± 0.2	6.3 ± 0.1	nd	17.3
Domaine de l'Esclade (grapes: Cabernet Sauvignon, Cabernet Franc)	France	8.4 ± 0.1	8.6 ± 0.1	<1.2	17.1
Chianti Ruffini (grapes: mainly Sangiovese)	Italy	7.2 ± 0.0	9.6 ± 0.0	nd	16.8
Côtes du Rhône Laurus, 1997 (grapes: Grenache)	France	8.5 ± 0.1	4.5 ± 0.1	<1.2	13.0
Chateuneuf-du-Pape Le Moulin Teyroud Réserve (grapes: Grenache, Mourvèdre, Syrah)	France	6.2 ± 0.0	2.1 ± 0.0	nd	8.3
white wines					
Gallo Sonoma Chardonnay, 1995	California	nd	nd	nd	0.0
Moselland Classic Riesling Trocken, 1998	Germany	nd	nd	nd	0.0

[a] Mean of triplicate assays ± SEM; nd, not detectable.

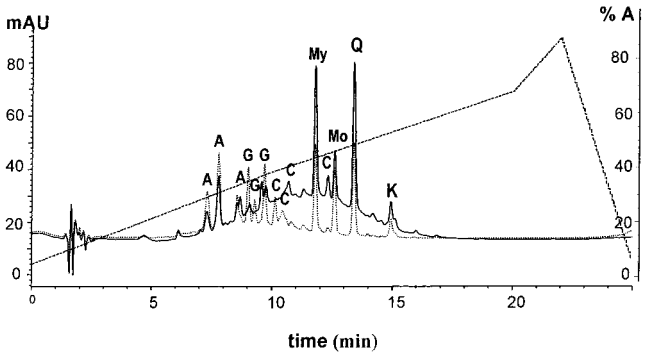

Figure 1. HPLC chromatograms of black currant wine 4 showing the separation of flavonols after hydrolysis by 0.3 (- - -) and 1.2 M (—) HCl (detected at 360 nm). Peaks: A, anthocyanin; G, flavonol glycoside; C, hydroxycinnamic acid; My, myricetin; Mo, morin; Q, quercetin; K, kaempferol. The percentage of organic eluent A (acetonitrile) in the gradient run is shown on the right.

In calculations of the flavonol concentrations, the recoveries could be ignored because of the use of the internal standard (morin).

Reference Compounds. Myricetin was obtained from Fluka (Buchs, Switzerland) and quercetin, kaempferol, and morin were from Sigma Chemical Co. (St. Louis, MO). Purity and concentration of the standard solutions (in methanol) were checked by measuring the absorbances at 360 nm. The true concentrations of the standard solutions were calculated using molar absorptivities given by Geissmann (1962).

RESULTS AND DISCUSSION

The amounts of myricetin, quercetin, and kaempferol varied considerably among the berry wines ($n = 18$) and grape wines ($n = 18$) (Tables 1 and 2). The total concentration of these flavonols ranged from 6 to 46 mg L^{-1} (mean 20 mg L^{-1}, $n = 16$) in the red berry wines and from 4 to 31 mg L^{-1} (mean 15 mg L^{-1}, $n = 16$) in the red grape wines. Flavonol concentration was very low in the white berry wines, and no flavonols were detected in the white grape wines.

Berry Wines. Myricetin and quercetin were the main flavonols detected in the red berry wines. Also kaempferol was found in all the red berry wines studied, but the quantification level was exceeded only in two wines. In black currant wines (wines 1−6), the amount of myricetin ranged from 7.3 to 22.6 mg L^{-1} and that of quercetin from 3.1 to 11.9 mg L^{-1}. Similar levels were found also in wines 7−9 produced from black currant and red currant, strawberry, or red raspberry, i.e., berries with low contents of flavonols (Häkkinen et al., 1999b). The myricetin and quercetin contents of wines 11−15 made partly or totally from crowberry were lower than expected, considering that this berry is almost as rich in flavonols as black currant is (Häkkinen et al., 1999b). Wine 16 made from bog whortleberry (56%), strawberry (19%), crowberry (17%), and black currant (8%) contained high amounts of both myricetin (22.1 mg L^{-1}) and quercetin (24.3 mg L^{-1}). The high amount of quercetin in this wine comes from bog whortleberry, which contains almost 4 times more quercetin than black currant does (Häkkinen et al., 1999b). Among the wines studied, the highest total concentration (46.4 mg L^{-1}) of flavonols was analyzed in this wine.

Only quercetin was found in the white berry wines, 4.1 mg L^{-1} in white currant wine, and traces in gooseberry wine (Table 1). Since white currant does not contain myricetin or kaempferol (Häkkinen et al., 1999b), the absence of those flavonols in this wine was expected. Kaempferol is present in gooseberry (Häkkinen et al., 1999b) but could not be detected in gooseberry wine.

The large variation observed in the flavonol concentrations is in line with the results reported by Heinonen et al. (1998) on total phenolic contens of Finnish berry wines. In black currant wines ($n = 5$), the content of

total phenolics ranged from 520 to 1820 mg of GAE L^{-1}, in other red berry wines ($n = 19$) from 335 to 1270 mg L^{-1}, and in white wines made from green and white currants ($n = 3$) from 250 to 270 mg L^{-1}. Lehtonen et al. (1999) analyzed phenolic compounds in Finnish berry wines made from black currant alone or blended with bilberry, crowberry, or strawberry. In contrast to our study, they detected flavonols only in one black currant wine (quercetin, 18 mg L^{-1}).

Grape Wines. Myricetin and quercetin were the main flavonols also in the red grape wines, and low levels of kaempferol were detected in 9 wines. In Cabernet Sauvignon wines ($n = 7$), the amount of myricetin ranged from 3.5 to 14.6 mg L^{-1} and that of quercetin from <1.2 to 10.2 mg L^{-1} (Table 2). A similar variation was observed in Merlot wines and other red wines analyzed. An exceptionally high level of quercetin (19.4 mg L^{-1}) was detected in Brown Brothers Merlot wine from California, and among the grape wines studied, the highest total concentration of flavonols (31.2 mg L^{-1}) was analyzed in this wine.

Our results are consistent with those of Justesen and Knuthsen (1998) and Justesen et al. (1998) who quantified flavonols in 21 red wines. Eight wines were made from Cabernet Sauvignon grapes, 2 wines from Merlot grapes, and 11 wines from other more local grapes. The mean concentrations of myricetin and quercetin were 10 and 8 mg L^{-1}, respectively. The amount of myricetin ranged from 2.5 to 22 mg L^{-1} and that of quercetin from 1.6 to 17.7 mg L^{-1}. Four of the wines contained kaempferol, 0.8−2.1 mg L^{-1}. Hertog et al. (1993b) analyzed 4 red wines and found myricetin, 6.9−9.3 mg L^{-1}, and quercetin, 4.1−16 mg L^{-1}. The present results agree also with the results of Frankel et al. (1995) who analyzed 14 red wines made from different grapes and vintages. The concentrations of myricetin and quercetin were 0.0−17.8 and 2.1−17.1 mg L^{-1}, respectively. In an extensive study by McDonald et al. (1998), the levels of myricetin were 2.7−19.8 and 5.9−16.3 mg L^{-1} and the levels of quercetin were 1.6−29.0 and 9.7−23.0 mg L^{-1} in Cabernet Sauvignon ($n = 22$) and Merlot ($n = 8$) wines, respectively.

Two white grape wines were analyzed, a Chardonnay wine from California and a Riesling wine from Germany (Table 2). We could not detect any measurable amounts of flavonols in these wines, which is in agreement with previous studies (Hertog et al., 1993b; Frankel et al., 1995). However, in a study of 31 white wines Betés-Saura et al. (1996) found a small amount of quercetin-3-glucuronide (0.20−0.31 mg L^{-1}).

In the present study, myricetin was the most abundant flavonol; only three berry wines and five grape wines contained more quercetin than myricetin. In the study of Hertog et al. (1993b), among 6 red wines analyzed only one Chianti wine contained more quercetin than myricetin. However, 40 out of 65 red wines studied by McDonald et al. (1998) contained more quercetin than myricetin.

Free Flavonols. Direct HPLC separation without the extraction and hydrolysis steps has been used in the analyses of phenolics present in grape wines (Lamuela-Raventós and Waterhouse, 1994; Lamuela-Raventós et al., 1995; Goldberg et al., 1996, 1998) and in berry wines (Lehtonen et al., 1999). We also ran our wine samples without methanol extraction and acid hydrolysis, and examples of the runs are presented in Figures 2 and 3. According to McDonald et al. (1998), in grape wines

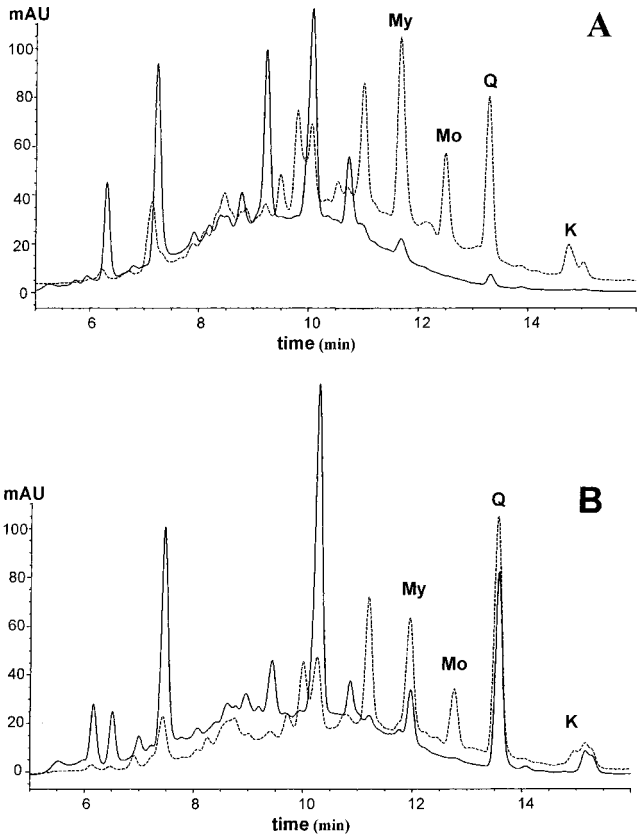

Figure 2. HPLC chromatograms of Sonoma County Carbenet Sauvignon (A) and Brown Brothers Merlot (B) wines showing the separation of flavonols with (- - -) and without (—) methanol extraction and acid hydrolysis (detected at 360 nm). Peaks are as in Figure 1.

considerable amounts of myricetin and quercetin are free, typically 20−50% of the total flavonol content. Also in most grape wines analyzed by us, variable amounts of free myricetin and quercetin were found (Figure 2). In berry wines, however, only unmeasurable amounts of free aglycons were detected (Figure 3).

Factors Affecting Flavonol Contents in Berry Wines. In accordance with the previous studies on grape wines (Hertog et al., 1993b; Frankel et al., 1995; Justesen and Knuthsen, 1998; McDonald et al., 1998), the amounts of the most important flavonols in wine, i.e., myricetin and quercetin, varied considerably in both the berry wines and grape wines analyzed by us. According to McDonald et al. (1998) and Goldberg et al. (1998), the concentrations of flavonols in wines are influenced by genetic factors of grapes, environmental conditions in the vineyard, and wine-processing techniques. The production of flavonol-rich wines is linked with the use of very ripe, thick-skinned grapes and with the favorable climatic conditions with respect to warmth, dryness, and sunshine. Higher flavonol content is connected also with the application of modern methods of vinification. Effective extraction of grapes and use of enzymatic hydrolysis during vinification and maceration produces wines with higher flavonol contents than do the traditional methods (McDonald et al., 1998). The genetic, environmental, and process technical factors are likely to affect the flavonol concentrations of berry wines also.

In wine-making, Öjebyn and Melalahti are the two main cultivars of black currant. Cv. Öjebyn has a thicker skin, which is advantageous for machine har-

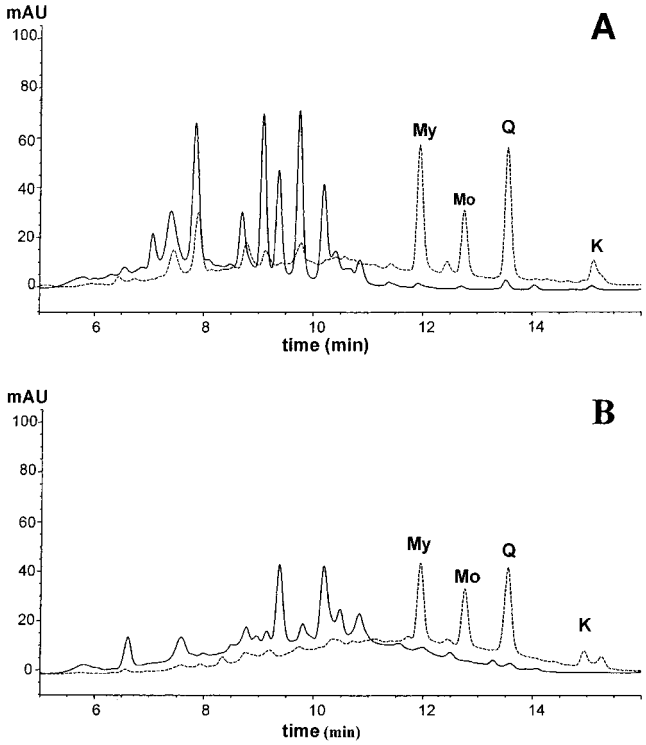

Figure 3. HPLC chromatograms of black currant wine 4 (A) and crowberry wine 15 (B) showing the separation of flavonols with (- - -) and without (—) methanol extraction and acid hydrolysis (detected at 360 nm). Peaks are as in Figure 1.

vesting, whereas the cv. Melalahti berries with thinner skins are smashed easily. By using the method described by Häkkinen et al. (1999b), we analyzed the flavonol contents of berries of these cultivars grown in the same area and collected on the same day: cv. Öjebyn contained myricetin, 140 mg kg^{-1}, and cv. Melalahti contained myricetin, 80 mg kg^{-1}. Quercetin concentrations were similar in these cultivars (84 and 86 mg kg^{-1}). These preliminary results indicate that the flavonol content of black currant wine may depend, in part, on the cultivar used and that the thicker-skinned cv. Öjebyn is preferable for the production of flavonol-rich wines. Unfortunately, information on the cultivars used for the production of the black currant wines analyzed in the present study was not available. McDonald et al. (1998) have shown that the red grape wines richest in flavonols are made from thick-skinned grapes such as Cabernet Sauvignon.

The low amount of flavonols in black currant/crowberry wines 11−13 could be the result of using unripe berries (producer's communication). It has been shown that in black currants the amounts of glycosides of quercetin and especially of myricetin increase during ripening (Herrmann, 1976). Since these results are from Germany and Russia, we also analyzed our black currants during the ripening season (late July to early September). In this preliminary experiment, the amounts of myricetin and quercetin increased by about 100 and 50%, respectively, during the first 2 weeks (data not shown). Therefore, the flavonol concentration probably is lower in wines made from berries harvested early in the season than in wines made from fully ripe berries.

A majority of the berry wines are produced from juice after horizontal pressing of frozen berries. Because several berry juices obtain a dark color, the maceration with skins and seeds has not been considered important, whereas for the production of red wine from grapes the maceration with all parts of crushed berries for 3−21 days is necessary (Soleas et al., 1997a). However, also some berry wine producers practice fermentation with berries (wines 1, 6, and 11−13).

During fermentation with grapes or berries, the polyphenols present in the skins are more effectively extracted into the wine. The localization of flavonols in black currants is not known. Therefore, we tentatively analyzed flavonols in whole black currant berries as well as in the skin and pulp separately. In fully ripe berries, about 96% of myricetin was found in the skin and only 4% in the pulp, whereas 34% of quercetin was in the skin and 66% in the pulp. These results suggest that fermentation with berries would improve the extraction of myricetin, and to a lesser extent that of quercetin, into the wine. The high myricetin content of black currant wine 1 (22.6 mg L^{-1}) might be a consequence of fermenting the wine with berries for 8 days. However, wines 6 and 11−13 fermented with berries for 3−7 days had low levels of myricetin.

In conclusion, the present data show that the flavonol contents of berry wines are comparable to those of grape wines. In wines made from black currants grown in Finland, the amounts of these polyphenols with multiple biological activities are similar to those in wines produced with modern vinification techniques from Cabernet Sauvignon or Merlot grapes grown in the warm and sunny climates of Australia, California, or Chile.

In addition to flavonols, berry wines also contain other phenolic compounds such as anthocyanins, catechins, hydroxycinnamic acids, and other phenolic acids (Lehtonen et al., 1999). Some of them probably are more abundant than flavonols. As already shown for grape wines (Frankel et al., 1995; Kerry and Abbey, 1997; Soleas et al., 1997b; Ghiselli et al., 1998), these substances are likely to make a significant contribution to the antioxidant activity and other biological activities of berry wines. The protective effects of berry wines on human health as well as the role of flavonols and other polyphenolic constituents remain to be elucidated.

ACKNOWLEDGMENT

We thank Mr. Ruud van Burgsteden for skillful technical assistance and the berry wine producers for providing the wines.

LITERATURE CITED

Betés-Saura, C.; Andrés-Lacueva, C.; Lamuela-Raventós, R. M. Phenolics in white free run juices and wines from Penedés by high-performance liquid chromatography: Changes during vinification. *J. Agric. Food Chem.* **1996**, *44*, 3040−3046.

Constant, J. Alcohol, ischemic heart disease, and the French Paradox. *Clin. Cardiol.* **1997**, *20*, 420−424.

Formica, J. V.; Regelson, W. Review of the biology of quercetin and related bioflavonoids. *Food Chem. Toxicol.* **1995**, *33*, 1061−1080.

Frankel, E. N.; Kanner, J.; German, J. B.; Parks, E.; Kinsella, J. E. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *Lancet* **1993**, *341*, 454−457.

Frankel, E. N.; Waterhouse, A. L.; Teissedre, P. L. Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. *J. Agric. Food Chem.* **1995**, *43*, 890−894.

Garcia-Closas, R.; Gonzalez, C. A.; Agudo, A.; Riboli, E. Intake of specific carotenoids and flavonoids and the risk of gastric cancer in Spain. *Cancer Causes Contr.* **1999**, *10*, 71−75.

Geissmann, T. A. *The Chemistry of Flavonoid Compounds;* Pergamon Press: London, Great Britain, 1962.

Ghiselli, A.; Nardini, M.; Baldi, A.; Scaccini, C. Antioxidant activity of different phenolic fractions separated from an Italian red wine. *J. Agric. Food Chem.* **1998**, *46*, 361−367.

Goldberg, D. M.; Tsang, E.; Karumanchiri, A.; Diamandis, E. P.; Soleas, G.; Ng, E. Method to assay the concentrations of phenolic constituents of biological interest in wines. *Anal. Chem.* **1996**, *68*, 1688−1694.

Goldberg, D. M.; Tsang, E.; Karumanchiri, A.; Soleas, G. J. Quercetin and p-coumaric acid concentrations in commercial wines. *Am. J. Enol. Vitic.* **1998**, *49*, 142−151.

Häkkinen, S.; Auriola, S. High-performance liquid chromatography with electrospray ionization mass spectrometry and diode array ultraviolet detection in the identification of flavonol aglycones and glycosides in berries. *J. Chromatogr. A* **1998**, *829*, 91−100.

Häkkinen, S. H.; Kärenlampi, S. O.; Heinonen, I. M.; Mykkänen, H. M.; Törrönen, A. R. HPLC method for screening of flavonoids and phenolic acids in berries. *J. Sci. Food Agric.* **1998**, *77*, 543−551.

Häkkinen, S.; Heinonen, M.; Kärenlampi, S.; Mykkänen, H.; Ruuskanen, J.; Törrönen, R. Screening of selected flavonoids and phenolic acids in 19 berries. *Food Res. Int.* **1999a**, *32*, 345−353.

Häkkinen, S. H.; Kärenlampi, S. O.; Heinonen, I. M.; Mykkänen, H. M.; Törrönen, A. R. Content of the flavonols quercetin, myricetin, and kaempferol in 25 edible berries. *J. Agric. Food Chem.* **1999b**, *47*, 2274−2279.

Heinonen, I. M.; Lehtonen, P. J.; Hopia, A. I. Antioxidant activity of berry and fruit wines and liquors. *J. Agric. Food Chem.* **1998**, *46*, 25−31.

Herrmann, K. Flavonols and flavones in food plants: a review. *J. Food Technol.* **1976**, *11*, 433−448.

Hertog, M. G. L.; Hollman, P. C. H. Potential health effects of the dietary flavonol quercetin. *Eur. J. Clin. Nutr.* **1996**, *50*, 63−71.

Hertog, M. G. L.; Feskens, E. J. M.; Hollman, P. C. H.; Katan, M. B.; Kromhout, D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *Lancet* **1993a**, *342*, 1007−1011.

Hertog, M. G. L.; Hollman, P. C. H.; van de Putte, B. Content of potentially anticarcinogenic flavonoids of tea infusions, wines, and fruit juices. *J. Agric. Food Chem.* **1993b**, *41*, 1242−1246.

Hertog, M. G. L.; Kromhout, D.; Aravanis, C.; Blackburn, H.; Buzina, R.; Fidanza, F.; Giampaoli S.; Jansen, A.; Menotti, A.; Nedeljkovic, S.; Pekkarinen, M.; Simic, B. S.; Toshima, H.; Feskens, E. J. M.; Hollman, P. C. H.; Katan, M. B. Flavonoid intake and long-term risk of coronary heart disease and cancer in the Seven Countries Study. *Arch. Intern. Med.* **1995**, *155*, 381−386.

Hertog, M. G. L.; Feskens, E. J. M.; Kromhout, D. Antioxidant flavonols and coronary heart disease risk. *Lancet* **1997**, *349*, 699.

Hollman, P. C. H.; van Trijp, J. M. P.; Buysman, M. N. C. P.; van der Gaag, M. S.; Mengelers, M. J. B.; de Vries, J. H. M. Relative bioavailability of the antioxidant flavonoid quercetin from various foods in man. *FEBS Lett.* **1997**, *418*, 152−156.

Justesen, U.; Knuthsen, P. The use of HPLC and mass spectrometry for the determination of flavonoids in red wines. *Polyphenol Communications 98, XIXth International Conference on Polyphenols;* 1998; pp 377−378.

Justesen, U.; Knuthsen, P.; Leth, T. Quantitative analysis of flavonols, flavones, and flavanones in fruits, vegetables and beverages by high-performance liquid chromatography with photodiode array and mass spectrometric detection. *J. Chromatogr. A.* **1998**, *799*, 101−110.

Keli, S. O.; Hertog, M. G. L.; Feskens, E. J. M.; Kromhout, D. Dietary flavonoids, antioxidant vitamins, and incidence of stroke. The Zutphen study. *Arch. Intern. Med.* **1996**, *156*, 637−642.

Kerry, N. L.; Abbey, M. Red wine and fractionated phenolic compounds prepared from red wine inhibit low-density lipoprotein oxidation in vitro. *Atherosclerosis* **1997**, *135*, 93−102.

Knekt, P.; Järvinen, R.; Reunanen, A.; Maatela, J. Flavonoid intake and coronary mortality in Finland: a cohort study. *Br. Med. J.* **1996**, *312*, 478−481.

Knekt, P.; Järvinen, R.; Seppänen, R.; Heliövaara, M.; Teppo, L.; Pukkala, E.; Aromaa, A. Dietary flavonoids and the risk of lung cancer and other malignant neoplasms. *Am. J. Epidemiol.* **1997**, *146*, 223−230.

Lairon, D.; Amiot, M. J. Flavonoids in food and natural antioxidants in wine. *Curr. Opin. Lipidol.* **1999**, *10*, 23−28.

Lamuela-Raventós, R. M.; Waterhouse, A. L. A direct HPLC separation of wine phenolics. *Am. J. Enol. Vitic.* **1994**, *45*, 1−5.

Lamuela-Raventós, R. M.; Romero-Pérez, A. I.; Waterhouse, A. L.; de la Torre-Boronat, M. C. Direct HPLC analysis of cis- and trans-resveratrol and Piceid isomers in Spanish red Vitis vinifera wines. *J. Agric. Food Chem.* **1995**, *43*, 281−283.

Lehtonen, P. J.; Rokka, M. M.; Hopia, A. I.; Heinonen, I. M. HPLC determination of phenolic compounds in berry and fruit wines and liqueurs. *Vitic. Enol. Sci.* **1999**, *54*, 33−38.

McDonald, M. S.; Hughes, M.; Burns, J.; Lean, M. E. J.; Matthews, D.; Crozier, A. Survey of the free and conjugated myricetin and quercetin content of red wines of different geographical origins. *J. Agric. Food Chem.* **1998**, *46*, 368−375.

Renaud, S.; de Lorgeril, M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet* **1992**, *339*, 1523−1526.

Soleas, G. J.; Diamandis, E. P.; Goldberg, D. M. Wine as a biological fluid: History, production, and role in disease prevention. *J. Clin. Lab. Anal.* **1997a**, *11*, 287−313.

Soleas, G. J.; Tomlinson, G.; Diamandis, E. P.; Goldberg, D. M. Relative contributions of polyphenolic constituents to the antioxidant status of wines: development of a predictive model. *J. Agric. Food Chem.* **1997b**, *45*, 3995−4003.

Received for review December 20, 1999. Revised manuscript received April 13, 2000. Accepted April 17, 2000. This study was financially supported by the Academy of Finland, the Jenny and Antti Wihuri Fund, and the Finnish Research and Information Center for Fruit Wines (EU Project 980544).

JF991388O