Flavonol Content Varies among Black Currant Cultivars

Tiina P. Mikkonen,[†,‡] Kaisu R. Määttä,[§] Anne T. Hukkanen,[†,‡] Harri I. Kokko,[†]
A. Riitta Törrönen,[#] Sirpa O. Kärenlampi,[†] and Reijo O. Karjalainen*,[‡,⊥]

Department of Biochemistry, Department of Ecology and Environmental Science, Department of
Physiology, and Department of Clinical Nutrition, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio,
Finland; and Agricultural Research Centre, Plant Protection, 31600 Jokioinen, Finland

Flavonoids and related plant compounds in fruits and vegetables are of particular importance as they have been found to possess antioxidant and free radical scavenging activity. The HPLC-based quantitative procedure, with improved extraction and hydrolysis, was used to analyze the content of the flavonols quercetin, myricetin, and kaempferol in 10 black currant cultivars from organic farms and in 5 cultivars from conventional farms. Myricetin was the most abundant flavonol, and its amount varied significantly among cultivars, from 8.9 to 24.5 mg 100 g^{-1} (fresh weight). The quercetin levels in black currant also varied widely among the cultivars, from 5.2 to 12.2 mg 100 g^{-1}. The kaempferol levels in black currant cultivars were low, ranging from 0.9 to 2.3 mg 100 g^{-1}. The sum of these major flavonols varied widely among black currant cultivars. No consistent differences in the contents of flavonols were found between the same black currant cultivars grown in organic and conventional ways. The high variability in the levels of flavonols in different cultivars offers possible avenues for identifying and selecting cultivars rich in certain flavonols for the special production of berries for industrial use.

Keywords: *Flavonoid; flavonol; quercetin; myricetin; kaempferol; black currant; cultivars; HPLC*

INTRODUCTION

Flavonoids are a large and diverse group of >4000 secondary plant products, comprising anthocyanins, flavonols, flavones, catechins, and flavanones (*1*). Many of these flavonoid compounds are known to play an important role in plants against microbial attacks (*2*), but more recent interest focuses on their potential health-promoting aspects in the human diet (*3, 4*). Epidemiological studies (*5, 6*) suggest that increased consumption of fruits and vegetables is often associated with a lower risk of degenerative diseases, such as cancer, cardiovascular disease, and immune and brain dysfunction. The high level of phenolic compounds possessing strong antioxidant and free radical scavenging activities are assumed to account for the beneficial effects of diets containing abundant amounts of fruits and vegetables (*7, 8*). Furthermore, in vitro oxidation model experiments for heart disease indicate that several plant flavonols, such as quercetin, myricetin, and rutin, are more powerful antioxidants than traditional vitamins (*8*). In addition, experimental data are accumulating that suggest specific antitumor properties of phenolic compounds such as quercetin (*9*). Little detailed information of major dietary sources of phenolics in different countries or about the absorption and metabolism of flavonoids following food consumption is available (*10*). Recently, Vinson et al. (*8*) listed the leading sources of phenols in the United States as tomato (41.7 mg/day), corn (37.0 mg/day), bean (30.2 mg/day) potato (27.6 mg/day), onion (19.8 mg/day), garlic (9.4 mg/day), carrot (6.8 mg/day), and broccoli (6.7 mg/day). It was estimated that the average per capita consumption of vegetable phenols in the United States was 218 mg/day of catechin equivalents, which is 3 times higher than the recommended intake of vitamin antioxidants.

Berries are another important source of potential health-promoting compounds. Blueberries (*Vaccinium* sp.*)* are currently used in many pharmaceutical and food supplement products in Europe and North America (*11*). Wild berries, such as bilberry, lingonberry, and cranberry, possess high levels of phenolic compounds, particularly flavonols, flavans, proanthocyanidins, and anthocyanins (*11–15*). Quercetin levels were highest in bog whortleberry (158 mg kg^{-1} fresh weight), lingonberry (74 and 146 mg kg^{-1} fresh weight), cranberry (83 and 121 mg kg^{-1} fresh weight), and bilberry (29–30 mg kg^{-1} fresh weight), and in cultivated berries, black currant and strawberry, the levels were significantly lower (44 and 7 mg kg^{-1}, respectively) (*14*). However, the level of myricetin was high in black currant (71 mg kg^{-1}) compared with the wild berries, bilberry (14 and 21 mg kg^{-1} fresh weight) and bog whortleberry (26 mg kg^{-1} fresh weight). In addition, black currant possesses a high content of vitamin C (120 mg g^{-1}), contributing thus to the high antioxidant activity of these berries (*14*).

Black currants are primarily grown for industrial use for making juices, jams, and yogurts. The potential health benefits of these products may be greatly increased if berries from cultivars with high contents of

* Address correspondence to this author at the Department of Ecology and Environmental Science, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland (telephone +358 17 163210; fax +358 17 163230; e-mail reijo.karjalainen@uku.fi).
† Department of Biochemistry, University of Kuopio.
‡ Department of Ecology and Environmental Science, University of Kuopio.
§ Department of Physiology, University of Kuopio.
# Department of Clinical Nutrition, University of Kuopio.
⊥ Agricultural Research Centre.

the health-promoting compounds are used as raw materials. However, very limited information is available about the phenolic content in black currant cultivars (*16*, *17*). Neither is much known about how cultivation practices, whether the berries are grown organically or in a conventional way, affect potential health-promoting compounds in black currant.

This study was designed to provide information the content of the flavonols myricetin, quercetin, and kaempferol in berries of black currant cultivars. Ten cultivars grown with organic practices and five cultivars grown with conventional practices were subjected to HPLC quantification of these flavonols.

EXPERIMENTAL PROCEDURES

Berry Samples. Berries from 10 organically cultivated black currant cultivars were harvested at the Research Station of the Agricultural Research Centre (Mikkeli, Finland) and from 5 cultivars conventionally grown on a farm close to the city of Kuopio, Finland. To obtain comparable samples, berries were selected visually and were at the same stage of berry development and from similar locations in the bushes. The berries were immediately frozen in liquid nitrogen and kept thereafter in dry ice until stored at −80 °C to analysis (from 2 to 3 weeks).

Extraction and Hydrolysis Conditions. The frozen berries (100 g) were crushed for 1 min in a food processor (Krups). Four parallel samples (5 g) were extracted with 50 mL of 50% aqueous methanol, containing 0.3 mg of morin as an internal standard and 20 mg of *tert*-butylhydroquinone (TBHQ) as antioxidant at 35 °C in a water bath for 2 h. For acid hydrolysis of the flavonol glycosides, the berry extract was centrifuged (10 min, 4000 rpm, 950*g*), and an aliquot (10 mL) was transferred into a glass tube; 2 mL of 6 M hydrochloric acid was added, and the mixture was heated in a water bath at 80 °C for 1 h. The hydrolyzed extracts were neutralized with 2 mL of 6 M sodium hydroxide, and flavonol aglycons were extracted into 20 mL of ethyl acetate in a roller mixer (Coulter Electronics, Ltd.) for 15 min. The upper ethyl acetate layer was evaporated to dryness in a vacuum evaporator in a 35 °C water bath. The residue was dissolved in 500 μL of methanol and filtered through a PVDF filter (0.45 μm pores) prior to analysis with HPLC.

HPLC Apparatus and Chromatographic Conditions. Samples were analyzed using an HPLC apparatus consisting of Hewlett-Packard (Waldbronn Analytical Division) 1100 quaternary pumps, an autosampler, and a diode array detector linked to an HP ChemStation data handling system. The reversed-phase separation was performed in LiChroCART column (125 × 3 mm, Purospher RP-18e, 5 μm particles, Merck, Darmstadt, Germany) protected by a respective integral guard column (4 × 4 mm, 5 μm particles). The flavonols were separated with gradient elution using 1% formic acid (A) and acetonitrile (B) as eluents. The elution system was as follows: 0−10 min, 5−40% of B in A (at a flow rate of 0.4 mL min^{-1}); 10−20 min, 40−70% of B in A (at a flow rate of 0.4 mL min^{-1}); 20−22 min, 70−90% of B in A (at a flow rate of 0.5 mL min^{-1}); 22−25 min, 90−5% of B in A (at a flow rate of 0.4 mL min^{-1}). Injection volume was 10 μL. Flavonol aglycons were detected at 360 nm and identified according to their retention times and UV spectra by comparing them with those of standards.

Reagents and Standards. Methanol and acetonitrile were of HPLC grade and purchased from Rathburn (Walkerburn, Scotland). Ethyl acetate (Lab-scan, Dublin, Ireland) was of analytical grade. Formic acid (98−100%, Merck), hydrochloric acid (minimum 37%, Riedel-de Haën, Seelze, Germany), and sodium hydroxide (FF Chemicals, Yli-Ii, Finland) were of pro analysis grade. The purity of TBHQ purchased from Aldrich (Steinheim, Germany) was 97%. Flavonol standards (purity percent determined by HPLC) quercetin (≥98%), kaempferol (97.7%), and morin (93.6%) were obtained from Sigma Chemi-
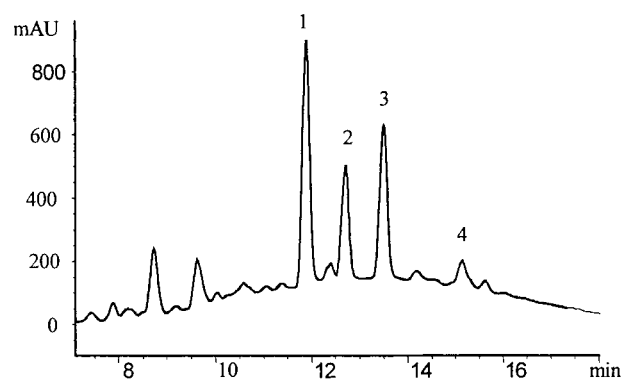

Figure 1. HPLC chromatogram for the quantification of flavonols in black currant berries: 1, myricetin; 2, morin; 3, quercetin; 4, kaempferol.

cal Co. (St. Louis, MO), and myricetin (97%) was from Fluka (BioChemika, Buchs, Switzerland). All standards were dissolved in methanol to an approximate concentration of 1 mg mL^{-1} as stock solutions (stored at −20 °C) for quantification of response factors. The internal standard morin (stored at 4 °C) was dissolved to 0.1 mg mL^{-1} in methanol prior to addition to berry samples. The exact concentrations of all standard solutions were determined by measuring the absorbances at 360 nm, using the values of molar absorptivities (log ϵ) given by Geissmann (*18*) (4.29 for myricetin, 4.32 for quercetin, and 4.15 for morin). For kaempferol the same value of molar absorptivity was used as for quercetin. For quantification of flavonols the corrected concentrations of all standards were used.

Validity of the Method and Quantification of the Flavonols. Contents of flavonols in berry samples were calculated from the chromatograms by using the concentration of the internal standard morin and sample peak areas. The response ratios between the quantified flavonols and morin were calculated using diluted stock solutions at the concentration range of 5−120 ng of flavonol μL^{-1}. The corresponding quantification limits were 0.3−6.3 mg 100 g^{-1}. The repeatability of the method was determined for four parallel samples by calculating the coefficients of variation (CVs). Recoveries for myricetin, quercetin, and kaempferol were measured by spiking pure standards into samples before extraction at two concentration levels (50 and 100% of the measured flavonol content). Because the recoveries determined using the internal standard were near 80% and the aim was to compare black currant cultivars with one another, the results were not corrected for recovery.

Statistical analyses were performed using the SAS (SAS Institute Inc., Cary, NC, 1989); differences among the means of cultivars were compared by least significant difference tests.

RESULTS

Recovery and Repeatibility Tests. The recoveries for pure aglycon standards were 67−68% (duplicate determination) for myricetin, 88−116% for quercetin, and 72−74% for kaempferol. The sensitivity of the method was good, and even kaempferol, a minor flavonol in black currant, was quantifiable. On average, the CVs for repeatability were 6.5, 5.1, and 4.9% for myricetin, quercetin, and kaempferol, respectively. The method was developed to recover the flavonols as aglycons in concentrated and stable form without interfering impurities (Figure 1).

Flavonol Contents in Berries of Black Currant Cultivars. Comparisons in the levels of flavonol contents revealed no consistent differences between black currants from conventional and organic cultivation practices. Data presented are for all cultivars. Data from

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Table 1. Content of Myricetin, Quercetin, and Kaempferol and the Sum of Those Three Flavonols in Berries of Organically and Conventionally Grown Black Currant Cultivars

cultivar	mg 100 g^{-1} fresh wt, mean ± SD			
	myricetin	quercetin	kaempferol	sum
Organically Grown				
Triton	24.5 ± 1.0	12.2 ± 0.4	1.7 ± 0.1	38.3 ± 1.4
local cv.	17.6 ± 0.5	11.5 ± 0.3	1.8 ± 0.01	30.9 ± 0.8
Ben Tron	20.3 ± 1.1	8.0 ± 0.3	2.3 ± 0.1	30.6 ± 1.5
Ojebyn	16.3 ± 0.8	9.6 ± 0.3	1.8 ± 0.1	27.8 ± 1.0
Titania	17.2 ± 1.0	8.1 ± 0.3	1.7 ± 0.1	27.0 ± 1.3
Hedda	12.2 ± 2.2	9.5 ± 0.8	1.8 ± 0.1	23.5 ± 2.9
Ola	8.9 ± 0.4	11.5 ± 0.5	2.1 ± 0.1	22.5 ± 1.0
Mortti	12.1 ± 0.8	6.9 ± 0.4	1.6 ± 0.1	20.5 ± 1.2
Melalahti	9.2 ± 0.7	9.0 ± 0.3	1.9 ± 0.1	20.1 ± 1.1
Hangaste	10.1 ± 0.3	7.0 ± 0.1	0.9 ± 0.02	17.9 ± 0.5
LSD5%	1.76	0.92	0.15	0.58
Conventionally Grown				
Ben Tron	15.4 ± 0.3	7.1 ± 0.1	2.2 ± 0.1	24.7 ± 0.3
Sunnia	14.0 ± 0.7	9.8 ± 0.6	1.8 ± 0.1	25.5 ± 1.2
Ojebyn	12.8 ± 1.1	10.4 ± 0.7	1.9 ± 0.1	25.1 ± 1.9
Intercontinental	11.3 ± 1.2	6.9 ± 0.6	1.2 ± 0.1	19.5 ± 1.7
Ben Alder	11.8 ± 0.9	5.2 ± 0.4	1.2 ± 0.1	18.2 ± 1.4

cultivars Ben Tron and Ojebyn, from both types of cultivation, are presented as averages.

In all black currants, myricetin was the predominant of the three flavonols studied, followed by quercetin (Table 1). Kaempferol was present in much smaller amounts (<5 mg/100 g). Quercetin levels were similar or exceeded those of myricetin in Melalahti and Ola (Table 1).

The amount of myricetin varied widely and significantly among black currant cultivars, being highest in cv. Triton (24.5 mg 100 g^{-1}) and lowest in cv. Ola (8.9 mg 100 g^{-1}). There were also significant differences between cultivars in the amount of quercetin. The highest quercetin content was found in cv. Triton (12.2 mg 100 g^{-1}), but quercetin content was very high also in the local variety and cv. Ola (11.5 mg 100 g^{-1} in both). The lowest quercetin content was found in the Finnish cultivars Mortti and Hangaste (6.9 and 7.0 mg 100 g^{-1}, respectively). The amount of kaempferol was very low in all cultivars (0.9–2.3 mg 100 g^{-1}), but significant cultivar-dependent variation could be seen (Table 1). The sums of the amounts of myricetin, quercetin, and kaempferol in black currant berries varied greatly between cultivars (Table 1). The highest content of these major flavonols was found in cv. Triton (38.3 mg 100 g^{-1}) and the lowest in Hangaste (17.9 mg 100 g^{-1}).

In conventionally grown black currants, the highest myricetin content was found in cv. Ben Tron (15.4 mg 100 g^{-1}) and the lowest in cv. Intercontinental (11.3 mg 100 g^{-1}). The amount of quercetin was highest in cv. Öjebyn (10.4 mg 100 g^{-1}) and lowest in cv. Ben Alder (5.2 mg 100 g^{-1}). The amount of kaempferol in these cultivars was very low (1.2–2.2 mg 100 g^{-1}). The sum of the three major flavonols was also highly variable among cultivars grown on a conventional farm (Table 1). The highest total flavonol content was found in cv. Sunniva (25.5 mg 100 g^{-1}) and the lowest in cv. Ben Alder (18.2 mg 100 g^{-1}).

DISCUSSION

The present data demonstrate that myricetin was the dominant flavonol in all cultivars except two (8.9–24.5 mg 100 g^{-1}), followed by quercetin (5.2–12.2 mg 100 g^{-1}) and kaempferol (1.2–2.2 mg 100 g^{-1}). Our results are in accordance to previous studies showing that black currants contain high amounts relative to other berries of myricetin and quercetin and a small amount of kaempferol (*13*, *15*). However, Häkkinen et al. (*15*) found higher quercetin than myricetin levels in black currant, whereas our data consistently show higher myricetin levels compared with quercetin. This difference may be due to the difficulty in quantifying myricetin, as this flavonol is unstable and sensitive to interference from other compounds (*19*). Additionally, flavonol amounts change greatly during fruit ripening, and the amount of myricetin is readily detectable only in fully ripe black currant berries (*17*). In the present study, the maturity stage of berries was evaluated visually, and all berries harvested were fully ripe.

The improved extraction procedure described in this study enabled the removal of a major part of the interfering compounds and allowed the sensitive detection of flavonol aglycons with high repeatability as revealed by low CVs between different samples. Methanol extraction prior to acid hydrolysis led to recoveries of pure aglycon standards of 67–68, 88–116, and 72–74%, for myricetin, quercetin, and kaempferol, respectively. In a previous study, Häkkinen et al. (*14*) found recoveries of 50 and 52% for myricetin and quercetin, respectively, but they detected no kaempferol in black currant berries. However, comparison of flavonoid contents observed in different studies should be done with caution as flavonoid content in berries is affected to a great extent by environmental conditions and by the maturity stage of the berries during sampling and extraction procedures. Furthermore, only in a few cases has the recovery been quantified, which should be a basic requirement for comparative analysis.

The data reported here demonstrate a high variability in the total and major flavonol content among black currant cultivars. Differences in the phenolic acid content among a few black currant cultivars have been previously found by Stöhr and Herrmann (*16*) and Starke and Hermann (*17*). Our data thus extend this observation and demonstrate high variability in the levels of myricetin and quercetin among 13 black currant cultivars. Black currants also contain high levels of other flavonoids, such as anthocyanins (*20*), so a more compherensive study is needed.

Cultivation practice, organic or conventional type of cultivation, does not seem to have major effects on flavonol contents in berries. There were no consistent differences among flavonol contents between berries grown either organically or conventionally. Apparently, the nutrition requirements of berries are only moderate, and the potential differences between mobilization of major nutrients into the berries, whether these are coming from fields of organically manured or fields supplemented with artificial fertilization, may have only minor impacts on the flavonol contents in black currants.

The increasing importance of functional ingredients in food provides new challenges for plant sciences to improve health-promoting phytochemicals in crop plants by crop husbandry, by plant breeding, and by genetic engineering. Assuming that the recent epidemiological and experimental studies are correct in suggesting that higher intakes of flavonoids from foods are associated with reduced risk of cancer, heart disease, and stroke, the immediate challenge is how to increase the levels of these beneficial phytochemicals in major food plants.

Flavonol Content in Black Currants

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The results presented in this paper indicate a high level of variability in the myricetin, quercetin, and kaempferol contents among black currant cultivars. This variation can be used to advantage to develop special cultivars high in flavonoids for target populations.

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