ANTIOXIDANTS & REDOX SIGNALING
Volume 3, Number 6, 2001
Mary Ann Liebert, Inc.

Forum Original Research Communication

Phenolic Compounds in Berries of Black, Red, Green, and White Currants (*Ribes* sp.)

KAISU MÄÄTTÄ,[1,4] AFAF KAMAL-ELDIN,[5] and RIITTA TÖRRÖNEN[2,3]

ABSTRACT

Multiple health benefits associated with phenolic compounds have raised the interest in the contents of these plant metabolites in foods. Several phenolic compounds were quantified from berries of *Ribes nigrum* (black and green currants) and *Ribes x pallidum* (red and white currants), by using sequential extraction with ethyl acetate and methanol and an optimized reversed-phase HPLC method with diode array detection. The highest contents of anthocyanins (3,011 mg/kg fresh weight, expressed as the aglycon) and flavonol glycosides (100 mg/kg) were found in black currant. The lack of anthocyanins in the colorless (green, white) berries was associated with increased levels of phenolic acids, especially *p*-coumaric acid (80 mg/kg in green currant vs. 45 mg/kg in black currant) and 4–hydroxybenzoic acid (18 mg/kg in white currant vs. 3 mg/kg in red currant). Previously, proanthocyanidins have not been quantified from berries. This study showed that the contents of extractable (22–41 mg/kg) and nonextractable proanthocyanidins (32–108 mg/kg) are comparable to those of other phenolics, with the exception of anthocyanins in black currant. Our results suggest that anthocyanins dominate in black and red currants, whereas proanthocyanidins and phenolic acids are the predominant phenolic compounds in green and white currants. Antioxid. Redox Signal. 3, 981–993.

INTRODUCTION

PHENOLIC COMPOUNDS are secondary plant metabolites widely distributed in the plant kingdom, and are also present in many foods and beverages of plant origin, *i.e.*, in fruits, vegetables, tea, and red wine (23, 36). During the past decade, flavonoids, phenolic acids, and related plant phenolics have received much attention due to their antioxidant, antimutagenic, anticarcinogenic, antiinflammatory, antimicrobial, and other biological properties and due to their plausible benefits on human health (8, 12, 22, 24, 26, 29, 33). Many of the protective effects of phenolic compounds have been ascribed to their antioxidant properties (24, 26, 28). In addition, they also affect several stages of signal transduction, including cell surface and intracellular receptors, intracellular mediators, kinases, the cell cycle, DNA replication-related enzymes, and gene expression (28).

Berry fruits are generally rich sources of phenolic compounds. The main subclasses of flavonoids and phenolic acids found in berries include hydroxybenzoic acids, hydroxycinnamic acids, flavonol glycosides, anthocyanins, flavan-3-ols, and their polymeric derivatives proanthocyanidins (Fig. 1) (23, 36). Very high

[1]Department of Physiology, [2]Department of Clinical Nutrition, and [3]Institute of Applied Biotechnology, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland.
[4]Finnish Research and Information Center for Fruit Wines, Hietapohjantie 926, FIN-73460 Muuruvesi, Finland.
[5]Department of Food Science, Swedish University of Agricultural Sciences (SLU), SE-750 07 Uppsala, Sweden.

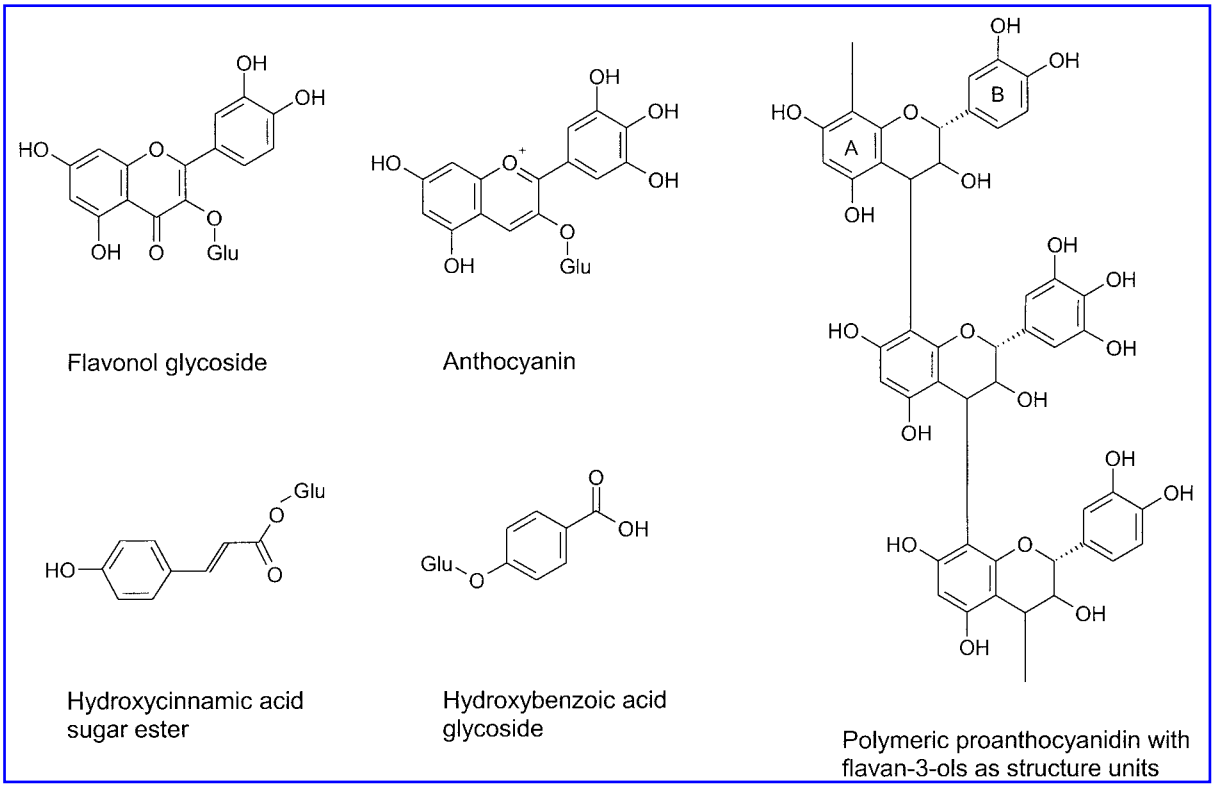

FIG. 1. Structures of the main subclasses of flavonoids and phenolic acids.

levels of anthocyanins are typically present in strongly colored berries, such as blueberry, bilberry, and black currant (5, 32). A majority of the 25 berries studied had a similar or higher level of flavonols (quercetin, myricetin, and kaempferol) than commonly consumed fruits or vegetables, with the exception of onions, kale, and some lettuces (11). Black currant was among the berries with the highest content of these flavonols. Berries are interesting food materials for use in delicious juices, wines, jams, jellies, ice creams, and cake toppings, and can be successfully utilized in the design of functional foods aiming to promote health. Qualitative and quantitative knowledge of the biologically active substances in berries and how they are affected by cultivation and processing is of significant importance to their utilization as functional ingredients in foods.

Currants [*Ribes* species (sp.); family Grossulariaceae, synonym Saxifragaceae] are commonly cultivated in home and commercial gardens in the cooler regions of Europe and North America. Black (*Ribes nigrum*) and red (*Ribes x pallidum*) currants are traditionally consumed as juices, jams, and jellies, and are also used for wine production. The less common unpigmented green and white variants evolved, due to genetic mutation, from black and red currants, respectively. Phenotypic observations suggest that anthocyanin pigments are not found in green and white currants, but nothing is known on whether this genetic mutation has effects on other phenolic components. This article presents the first systematic study of flavonoids and phenolic acids in berries of *R. nigrum* (black and green currants) and *R. x pallidum* (red and white currants). Phenolic compounds analyzed were hydroxybenzoic and hydroxycinnamic acid derivatives, flavan-3-ols, flavonol glycosides, anthocyanins, and proanthocyanidins.

MATERIALS AND METHODS

Berries

Berries were harvested ripe during August 2000 in the eastern part of Finland. Black (*R. nigrum* cv. Öjebyn) and green (*R. nigrum* cv.

Vertti) currants were obtained from a local berry farm in Kuopio, whereas red (*R. x pallidum* cv. Red Dutch) and white (*R. x pallidum* cv. White Dutch) currants were obtained from the Research Garden of the University of Kuopio. The berries were frozen at −20°C and analyzed within 2 days.

Standards

4–Hydroxybenzoic acid (H5376), vanillic acid (V2250), chlorogenic acid (C3878), *p*-coumaric acid (C9008), caffeic acid (C0625), ferulic acid (F3500), (+)-catechin (C1251), (−)-epicatechin (E1753), rutin (R5143), quercetin (Q0125), and kaempferol (K0133) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.), and myricetin was obtained from Fluka (Buchs, Switzerland). Delphinidin and cyanidin 3-*O*-β-glucopyranosides were obtained from Polyphenols AS (Sandnes, Norway), and delphinidin (0904S) and cyanidin (0909S) chlorides were purchased from Extrasynthese (Geney Cedex, France). The standards were dissolved in methanol to a concentration of ~1 mg/ml and stored at −20°C as stock solutions.

Solvent extraction

Frozen berries (100 g) were homogenized, and samples (5 g, except black currant 3 g) were weighed in centrifuge tubes for extraction. All extractions and further analyses were performed in quadruplicate. A stepwise, two-phase solvent extraction procedure was used for the extraction of phenolic compounds (Fig. 2). Conjugated forms of phenolic acids, flavan-3-ols, flavonol glycosides, and part of the extractable proanthocyanidins were first extracted into ethyl acetate (4 × 10 ml) with intermittent mixing and centrifugation. The berry matrix was acidified with hydrochloric acid (HCl) (2 *M*, 2 ml), and anthocyanins, extractable proanthocyanidins, and residues of the previously extracted phenolic compounds were extracted into methanol (20 ml + 3 × 10 ml). Separately, the ethyl acetate extract and an aliquot of the methanol extract (10 ml) were evaporated to dryness and reconstituted into methanol (1 ml). Only anthocyanins of black currant could be analyzed in the methanol extract without concentration.

Acid hydrolysis

The methanolic solutions of both extracts and the berry residue were acidified by adding concentrated HCl to a final concentration of 0.6 *M*. The ethyl acetate extract was kept in a boiling water bath for 5 min, and the methanol extract and the residue were refluxed (60°C) for 2 h. Upon heating in acidic methanol, glycosylated phenolic compounds were deconjugated to aglycons, hydroxycinnamic acid esters were converted to corresponding methyl esters (31), flavan-3-ols were decomposed, and proanthocyanidins were converted to anthocyanidins (Fig. 2). As the conversion of proanthocyanidins to anthocyanidins by acid treatment was reported to be only partial (30), the conditions of acid hydrolysis were optimized, using green currant, to provide the highest possible yield of anthocyanidins.

High-performance liquid chromatography (HPLC) analysis

All samples were filtered through a 0.45-μm Regen Cellulose syringe filter (TITAN, Gloucester, U.K.) prior to analysis. The HPLC apparatus consisted of a Hewlett–Packard (Waldbronn Analytical Division, Germany) instrument with a 1100 series quaternary pump, an autosampler, and a diode array detector linked to an HPChemStation data handling system. Phenolic compounds were separated on a LiChroCART Purospher RP-18e column (125 × 3 mm i.d., 5 μm; Merck, Darmstadt, Germany) protected with a guard column of the same material (4 × 4 mm). For the analysis of hydroxybenzoic and hydroxycinnamic acid derivatives, flavan-3-ols, and flavonol glycosides, as well as their deconjugated forms (after acid hydrolysis) and anthocyanidins, a 20-min linear gradient from 5 to 30% acetonitrile in 1% formic acid was used at a flow rate of 0.5 ml/min. For the analysis of anthocyanins, a gradient of acetonitrile in 5% formic acid was used. The concentration of acetonitrile was as follows: 0–5 min, 5–10%; 5–10 min, 10% (isocratic separation); 10–25 min, 10–40% (flow rate 0.5 ml/min). Both gradients were followed by

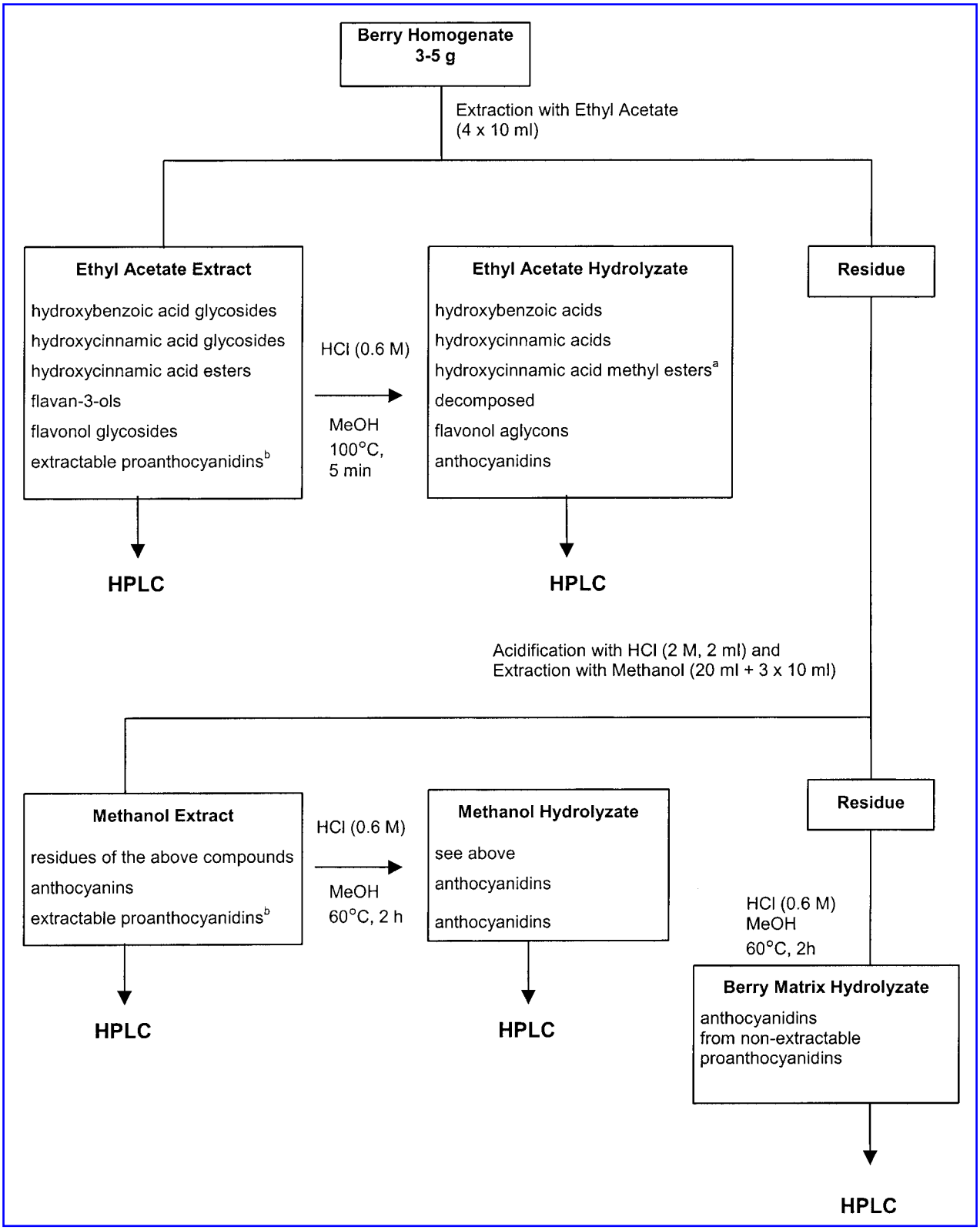

FIG. 2. Scheme of the analytical methods. [a]Known deconjugation products in the methanolic acid hydrolysis (31). [b]Ethyl acetate extracts contain low molecular weight proanthocyanidins (dimers, trimers, tetramers, etc.), and methanol extracts consist of more polymeric proanthocyanidins (38).

washing the column by increasing the concentration of acetonitrile to 90% in 10 min, by returning to the initial conditions in 5 min, and by reequilibration of the column.

Diode array detection from 240 to 520 nm was used for the identification of the phenolic compounds. Peak assignment of the conjugated forms of phenolic compounds in the chromatograms was based on comparison of their spectral characteristics with those of the representative standards of the phenolic subclasses (Table 1). Retention times were used for identification of peaks with spectra matching with authentic standards. Phenolic acid derivatives were divided into hydroxybenzoic (4-hydroxybenzoic and dihydroxy/methoxybenzoic) acid derivatives and hydroxycinnamic (*p*-coumaric and caffeic/ferulic) acid derivatives according to their spectral characteristics. Purity of the peaks was monitored by checking the spectra at the leading edge, apex, and tailing edge of the peaks. The occurrence of phenolic acid glycosides and flavonol glycosides was verified after acid hydrolysis by comparing the retention times and spectral characteristics of aglycons with those of available standards.

Conjugated forms of phenolic compounds were quantified for the weight of the aglycon using response factors of representative standards near their characteristic wavelengths of maximum absorption (Table 1): hydroxybenzoic acids at 260 nm, hydroxycinnamic acids at 320 nm, flavonol glycosides at 360 nm, and anthocyanins and proanthocyanidins as anthocyanidins at 520 nm. Response factors of anthocyanins and anthocyanidins were determined in acidified (0.6 *M* HCl) methanol. Hydrolysis products of proanthocyanidins included two unknown peaks absorbing at 520 nm, which were quantified using response factors of cyanidin and delphinidin. Possible proanthocyanidins in the methanol extract of pigmented currants were not quantified because of the co-occurrence of the same deconjugation products of anthocyanins after acid hydrolysis (Fig. 2).

RESULTS

In this study, a sequence of solvent extraction and acid hydrolysis methods was used to allow reasonable estimation of composition and contents of several flavonoids and phenolic acids in currants. The extractability and products of acid hydrolysis after each extraction step are presented as a scheme in Fig. 2. Samples were analyzed by HPLC using two gradient systems, one for the analysis of hydroxybenzoic and hydroxycinnamic acid derivatives, flavan-3-ols, flavonol glycosides (Fig. 3) as well as their deconjugated forms and anthocyanidins, and the other for the analysis of anthocyanins (see Fig. 5). The compounds were quantified near their characteristic absorption

Table 1. Spectral Characteristics of the Representative Standards for Phenolic Subclasses in HPLC-DAD* from 240 to 520 nm

		Spectral characteristics	
Subclass	*Representative standard(s)*	*Wavelength of maximum absorption (nm) and shoulders (sh)*	*Range of absorption (nm)*
Hydroxybenzoic acids	4-Hydroxybenzoic acid	256	240–290
	Vanillic acid	262, 292	240–310
Hydroxycinnamic acids	*p*-Coumaric acid	300sh, 310	240–360
	Caffeic/ferulic acid	300sh, 326	240–380
Flavan-3-ols	(+)-Catechin	278	240–300
	(−)-Epicatechin	278	240–300
Flavonols	Rutin (quercetin 3-rutinoside)	254, 300sh, 354	240–420
Anthocyanins	Delphinidin 3-*O*-glucoside	278, 524	240–310, 440–550
	Cyanidin 3-*O*-glucoside	278, 516	240–300, 430–550
Anthocyanidins	Delphinidin	274, 528	240–290, 450–550
	Cyanidin	274, 524	240–290, 450–550

*DAD, diode array detection.

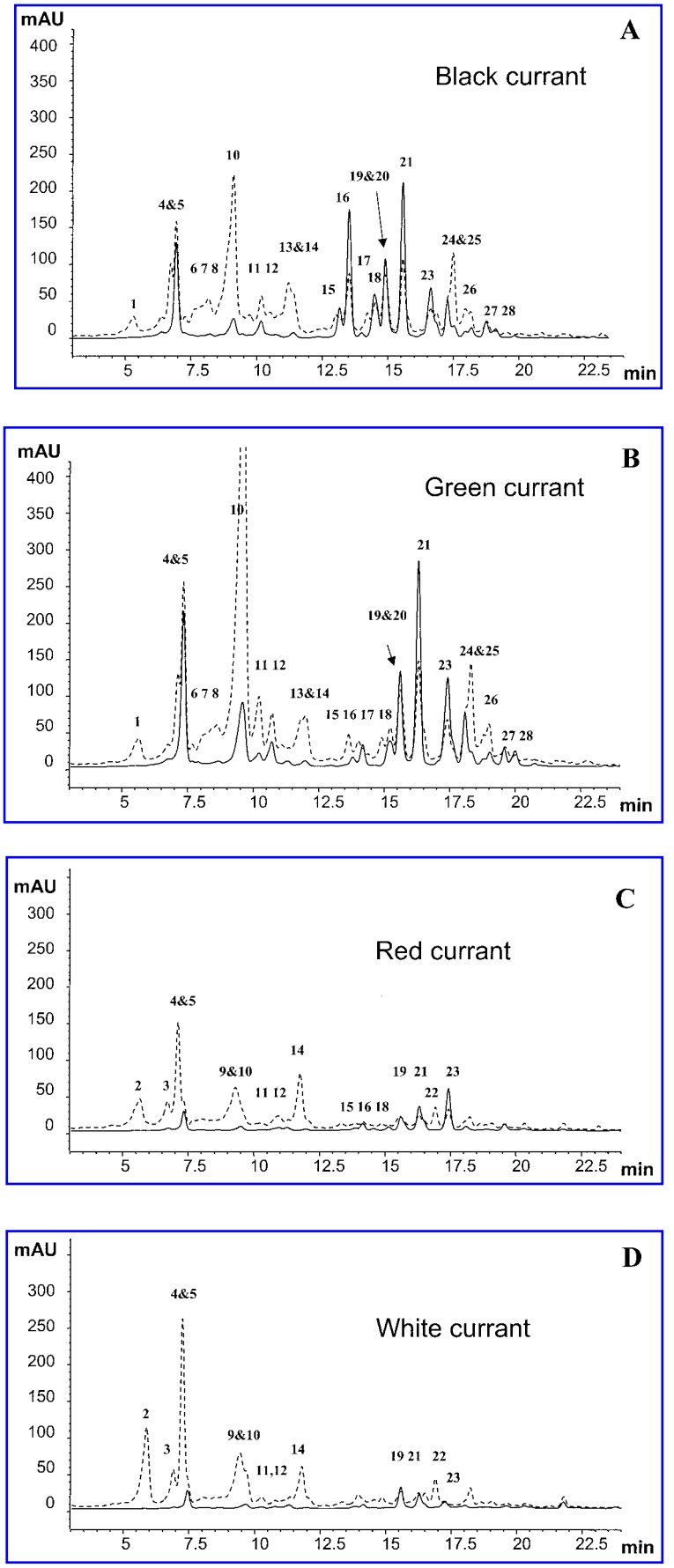

FIG. 3. Chromatographic patterns at 360 nm (solid line) and 280 nm (dotted line) of ethyl acetate-extractable phenolic compounds. Peak identification in the order of retention: (1, 4, 14, 17, 22) not identified; (2) 4-hydroxybenzoic acid derivative; (3) dihydroxy/methoxybenzoic acid derivative; (9) (+)-catechin; (13) (−)-epicatechin; (5, 12, 20, 26) caffeic/ferulic acid derivative; (6, 7, 8, 10, 11, 25) *p*-coumaric acid derivative; (19) rutin; (15, 16, 18, 21, 23, 24, 27, 28) other flavonol glycosides.

maxima using response factors of authentic standards. Because some compounds were extracted in both ethyl acetate and methanol, results were combined for quantification. As the concentration of phenolic compounds is known to vary with season and a wide range of environmental factors such as climate and soil condition (23), related black and green currants as well as red and white currants analyzed in this study were harvested from the same field to minimize variations due to these factors.

Hydroxybenzoic acid derivatives

Schuster and Herrmann (35) reported the presence of 4–hydroxybenzoic acid (a monohydroxybenzoic acid) and protocatechuic acid (a dihydroxybenzoic acid) as glucosides in black and red currants. In the present study, however, peaks with spectral characteristics similar to those of mono- or dihydroxybenzoic acid derivatives could not be identified in black and green currants (Fig. 3A and B, respectively). In red and white currants (Fig. 3C and D, dotted lines, respectively), two peaks (2 and 3) exhibiting spectral characteristics similar to those of 4–hydroxybenzoic acid and vanillic acid (a hydroxymethoxybenzoic acid) derivatives were detected at 280 nm. The presence of 4–hydroxybenzoic acid in these berries was verified after acid hydrolysis of the ethyl acetate extract. Unfortunately, this verification procedure was not possible for vanillic acid, because some unknown decomposition product(s) co-eluted and markedly affected the spectrum of the peak. The identity of the dihydroxybenzoic acid derivative (Fig. 3C and D, peak 3) in red and white currants is not clear using this data because vanillic acid has the same spectral characteristics as many other benzoic acids substituted with two adjacent hydroxy and/or methoxy groups (2). The content of 4–hydroxybenzoic acid was higher in white currant than in red currant (Table 2). The contents of hydroxybenzoic acid derivatives in red and white currants are in close agreement with those published in the literature (35, 37).

Hydroxycinnamic acid derivatives

The wavelength of maximum absorption of hydroxycinnamic acids is near 320 nm, but these compounds also absorb appreciably at 280 nm (Fig. 3, dotted lines). Free hydroxycinnamic acids were not found in any of the currants studied. Peaks were tentatively assigned as *p*-coumaric acid derivatives or as caffeic and/or ferulic acid derivatives by comparison with the spectra of the respective aglycon standards. The retention times of the hydroxycinnamic acid derivatives ranged from 7 to 20 min (Fig. 3, dotted lines), indicating that these compounds are present as derivatives of different degrees of polarity. The wavelengths of maximum absorption of the detected hydroxycinnamic acid derivatives were shifted 4 nm to longer wavelengths compared with the free standards.

After acid hydrolysis of the ethyl acetate extracts, two major new nonpolar peaks were obtained, with spectral characteristics similar to those of *p*-coumaric acid and caffeic/ferulic

Table 2. Contents of Phenolic Compounds in Black, Green, Red, and White Currants (mg/kg Fresh Weight, Mean ± SE)

	Hydroxybenzoic acid derivatives		*Hydroxycinnamic acid derivatives*			*Anthocyanins*		*Proanthocyanidins (PC:PD)*	
	HBA	*diHBA*	p-*CA*	*C/FA*	*Flavonol glycosides*	*Del-gly*	*Cy-gly*	*Extractable*	*Nonextractable*
Black currant	ND	ND	45 ± 1	36 ± 0	100 ± 2	1,518 ± 42	1,493 ± 38	NA	108 (49:51)
Green currant	ND	ND	80 ± 3	40 ± 2	77 ± 4	ND	ND	41 (38:62)	50 (40:60)
Red currant	3 ± 0	3 ± 0	3 ± 0	5 ± 0	8 ± 0	ND	177 ± 6	NA	34 (14:86)
White currant	18 ± 1	5 ± 1	9 ± 1	13 ± 1	6 ± 0	ND	ND	22 (15:85)	32 (11:89)

SE, standard error for four parallel samples; PC, procyanidin units; PD, prodelphinidin units; PC:PD, procyanidin/prodelphinidin ratio; HBA, 4-hydroxybenzoic acid; diHBA, dihydroxy(methoxy)benzoic acid; *p*-CA, *p*-coumaric acid; C/FA, caffeic/ferulic acid; Del-gly, delphinidin glycosides; Cy-gly, cyanidin glycosides; ND, not detected; NA, not analyzed.

acids. These peaks may represent the methyl ester derivatives formed in the methanolic acid hydrolysis of hydroxycinnamic acid esters (31). Schuster and Herrmann (35) reported the presence of hydroxycinnamic acids as glucosides and as quinic acid and glucose esters in black and red currants.

The contents of hydroxycinnamic acid derivatives were higher in black and green currants than in red and white currants (Table 2). The contents of caffeic/ferulic acid derivatives were similar in black and green currants (36 and 40 mg/kg fresh weight, respectively), but the content of *p*-coumaric acid derivatives was almost doubled by the mutation from black (45 mg/kg) to green (80 mg/kg) currant. In white currant, however, the contents of both *p*-coumaric acid and caffeic/ferulic acid derivatives were higher than in red currant. Values obtained in this study seem to be lower than in the literature (35, 37). The probable explanations for this inconsistency may be due to differences in the methods used for extractions, hydrolysis, and quantification.

Flavan-3-ols

Figure 3 (dotted lines) also shows peaks due to flavan-3-ols, two of which were assigned as (+)-catechin (peak 9) and (−)-epicatechin (peak 13) because they have the same retention times as the authentic standards. The presence of (+)-catechin was confirmed, by comparison of ultraviolet (UV) spectra, in red and white currants but not in black and green currants, where a considerable amount of a *p*-coumaric acid derivative, with strong UV absorption, co-eluted and overlapped with the possible (+)-catechin. Figure 4 shows the spectrum of standard (+)-catechin compared with that obtained for the ethyl acetate extract of red currant with broadening of the UV spectrum due to co-elution of the *p*-coumaric acid derivative.

In our chromatography, the unknown peak 14 overlapped with (−)-epicatechin, disturbing its quantification. It was, however, possible to detect (−)-epicatechin in green and black but not in white and red currants. This observation is consistent with a recent study of flavan-3-ol contents in plant foods (1), where (−)-epicatechin was not detected in red and white currants. In the present study, it was not possible to quantify flavan-3-ols either in the unhydrolyzed extracts because of the above-mentioned contamination of peaks or in the acid-hydrolyzed extracts due to decomposition of their labile structure in hot acid treatment (3).

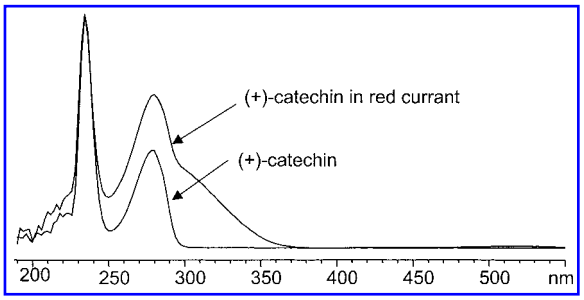

FIG. 4. Combined spectrum of (+)-catechin and a *p*-coumaric acid derivative in red currant (Fig. 3, peaks 9 and 10) compared with the standard.

Flavonol glycosides

For the detection of flavonol glycosides, chromatograms were recorded at 360 nm (Fig. 3, solid lines). Free flavonol aglycons were not found in any of the currants. Flavonol glycosides eluting at the same retention times were found in all currants studied, although in different proportions. After acid hydrolysis of the ethyl acetate extracts, quercetin and kaempferol aglycons were found in black and green currants, but an overlapping unknown deconjugation product of the caffeic/ferulic acid derivative(s) disturbed the distinctive spectral identification of myricetin. Fortunately, the absorption of flavonol aglycons extends to longer wavelengths (420 nm) than that of caffeic/ferulic acid derivatives (380 nm) (Table 1). Therefore, absorption at 420 nm at the retention time of standard myricetin (18.8 min) verified its presence in black and green currants. Studies on identification of peaks is ongoing using liquid chromatography/mass spectrometry. In red and white currants, quercetin aglycon was identified after acid hydrolysis, but the peaks at the retention times of myricetin and kaempferol were below the limit for reliable spectral identification. Red and white currants contained very low levels of flavonol glycosides compared with green and black currants (Table 2). The contents of

flavonol glycosides obtained in this study are comparable to those reported before, although the previous methods were based on the analysis of flavonol aglycons after acid hydrolysis (11, 18).

Anthocyanins

As previously mentioned, anthocyanins (anthocyanidin glycosides) are responsible for the typical black and red pigments of the respective currants, whereas green and white currants lack anthocyanin pigments. In agreement with Goiffon *et al*. (9), the typical anthocyanins were four glucoside and rutinoside derivatives of cyanidin and delphinidin in black currant (Fig. 5A) and five different glycosides of cyanidin in red currant (Fig. 5B). Free anthocyanidins were not found in any of the currants studied. Black currant had a very high content of anthocyanins (3,011 mg/kg fresh weight) compared with red currant (177 mg/kg) (Table 2). The contents and identity of anthocyanins are in agreement with previous reports on black currant (5, 16, 17) and red currant (5, 27).

Proanthocyanidins

Strong purple colors were formed in the colorless methanol extracts of white and green currants and in the lightly reddish berry residues of the four currants upon acid hydrolysis due to release of anthocyanidin pigments from colorless proanthocyanidins. This phenomenon was utilized for tentative quantification of proanthocyanidins as anthocyanidins (cyanidin and delphinidin) from the extracts (extractable proanthocyanidins) and from the residues (nonextractable proanthocyanidins). Of the extractable proanthocyanidins, the majority was found in the methanol extract and only a minor proportion (2–6%) was extracted into ethyl acetate.

Proanthocyanidins can be defined by their

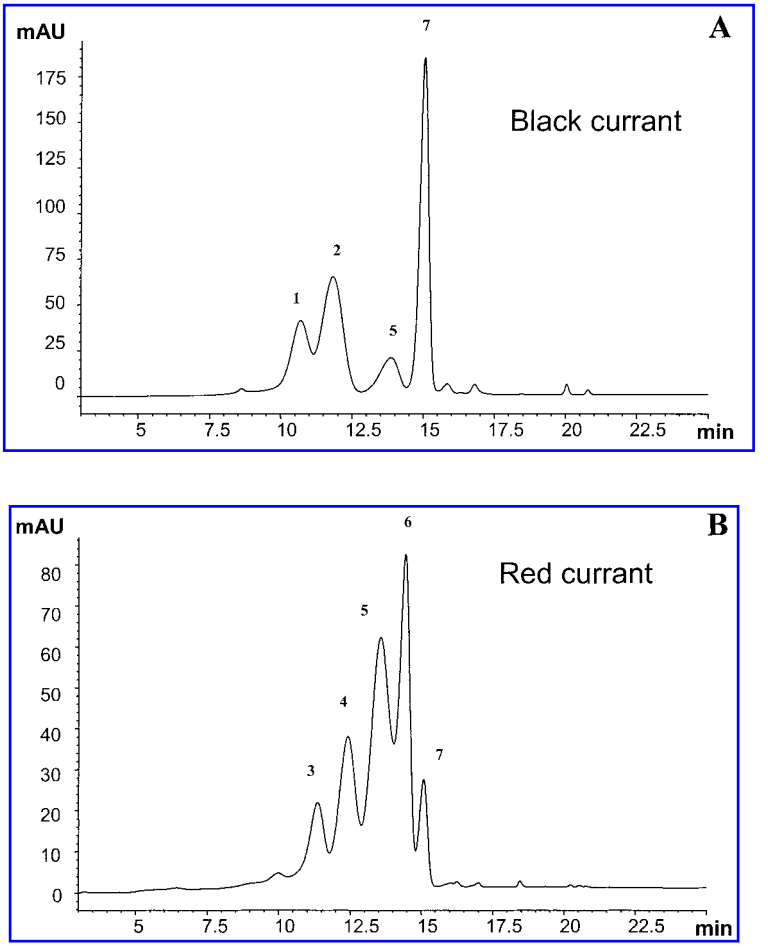

FIG. 5. Chromatographic patterns at 520 nm of anthocyanins in black and red currants. Peak identification in the order of retention: (1) delphinidin 3-*O*-rutinoside; (2) delphinidin 3-*O*-glucoside; (3, 4, 6) cyanidin glycosides; (5) cyanidin 3-*O*-rutinoside; (7) cyanidin 3-*O*-glucoside.

procyanidin/prodelphinidin ratio. Procyanidins are composed mainly of (+)-catechin and (−)-epicatechin units (Fig. 1, dihydroxylation in the B ring), and prodelphinidins of (+)-gallocatechin and (−)-epigallocatechin units (Fig. 1, trihydroxylation at the B ring) (7). There was a similarity in the procyanidin/prodelphinidin ratios of the nonextractable proanthocyanidins between red and white currants, and between black and green currants (Table 2). Moreover, the procyanidin/prodelphinidin ratios in the extractable and nonextractable forms resembled each other in green and white currants. The present data are comparable to previous results (7).

As anthocyanins deconjugate to anthocyanidins by acid hydrolysis, it was not possible to analyze extractable proanthocyanidins from the methanol extracts of black and red currants. The amounts of the nonextractable proanthocyanidins were comparable in red and white currants, but black currant exhibited double the amount of proanthocyanidins in green currant. Black and green currants contained more extractable and nonextractable proanthocyanidins than red and white currants.

DISCUSSION

Previously, the contents of anthocyanins in juices and extracts of black currant and other pigmented berries have been quantified as part of studies of their antioxidant capacity (5, 25, 32, 39) or to monitor changes during processing and storage (17, 20). Phenolic profiles (comprising phenolic acids and flavonols only) (10) and contents of flavonol aglycons have been determined for black, green, red, and white currants (11, 15, 18). The contents and compositions of proanthocyanidins have been studied for fruits such as grapes, apples, and pears (for review, see 34), but not for berry fruits. In the present study, the analytical methods extended for the estimation of proanthocyanidins showed that the contents of extractable and nonextractable proanthocyanidins in berries of currants are comparable to those of other phenolics, with the exception of anthocyanins in black currant (Table 2).

This study shows that black currant contains by far higher amounts of phenolic antioxidants, mainly anthocyanins, than the other currants. A total of ~3,000 mg/kg fresh weight anthocyanins was obtained in the black currant samples analyzed in this study. Previously, high contents of anthocyanins (mg/kg fresh weight, expressed as aglycon) were found in black currant (1,500–3,400 mg/kg) (5) as well as in other strongly pigmented berries such as blueberry (400–1,200 mg/kg) and bilberry (2,000 mg/kg) (32), whereas clearly lower levels have been reported for berries with lighter red color including red currant (100–200 mg/kg), red raspberry (200–500 mg/kg) (5), and strawberry (150–280 mg/kg) (39). The daily intake of flavonoids in the western countries was estimated to be 0.5–1.0 g, of which ~0.2 g was anthocyanins (21). Consumption of black currant and other dark-colored berries may significantly contribute to the dietary intake of anthocyanins.

In studies on juices of yellow to black pigmented raspberry species (6), extracts of a wide range of blueberry species and cultivars (32), and extracts of blackberry, raspberry, strawberry, and blueberry (20, 39), high antioxidant activities were found to depend not only on the content of anthocyanins, but also on the content of total phenolics. In studies with berries with strong colors compared with berries with light colors, significant differences in the antioxidant activities were found in one study (13), but not in another (19). As one explanation, the authors related this difference to the different techniques used in the preparation of the berry extracts and, consequently, to differences in profiles of the active phenolic compounds (19).

Anthocyanins and other phenolic compounds have a physiological role in fruits (23), functioning in the chemical defense system against environmental stress factors (4). It might, therefore, be possible that the lack of anthocyanins in green and white currants is compensated by increased levels of other phenolics in a way that can be explained by the known routes of biosynthesis. Phenolic compounds originate from phenylalanine, which is deaminated to cinnamic acid, the precursor of hydroxybenzoic and hydroxycinnamic acids. Cinnamic acid is hydroxylated to *p*-coumaric acid, which can be converted to *p*-coumaroyl-

CoA, which is the key intermediate to the synthesis of flavonols, anthocyanins, flavan-3-ols, and proanthocyanidins (14).

In the present study, the content of *p*-coumaric acid was higher in green than in black currant, and the contents of 4-hydroxybenzoic acid and hydroxycinnamic acids were higher in white than in red currant (Table 2). These results suggest that the lack of anthocyanins is associated with increased levels of phenolic acids. In a previous study with grapes, however, the relative proportions of *p*-coumaroyltartaric esters decreased, but those of the caffeic and ferulic acid derivatives increased with the change from colored to white forms (23). Correlations between different phenolic compounds and how they are influenced by the phenotype of species, as well as maturity and environmental conditions, need to be studied in a statistically sound model.

Developments in analytical methods are important for studies aiming to quantify and estimate the dietary intake of phenolic compounds, which are believed to be good for health. Complete analysis of phenolic compounds in berries is complicated by the variety of conjugation forms in which these compounds are present. In this study, we analyzed a wide variety of phenolic compounds using sequential extraction with ethyl acetate and methanol followed by methanolic acid hydrolysis and HPLC analyses. This method needs to be developed further for simplification, identification of unknown peaks, and reliable quantification of conjugates, especially proanthocyanidins. Until then, the results presented in this article may be considered as the best approximation for the levels of phenolic compounds in currants. Our results suggest that anthocyanins dominate in black and red currants, whereas proanthocyanidins and phenolic acids are the predominant phenolics in green and white currants.

CHEMICAL NAMES

4-Hydroxybenzoic acid [Chemical Abstract Service (CAS) 99-96-7]; vanillic acid (4-hydroxy-3-methoxybenzoic acid, CAS 121-34-6); *p*-coumaric acid (4-hydroxycinnamic acid, CAS 7400-08-0); ferulic acid (3-methoxy-4-hydroxycinnamic acid, CAS 1135-24-6 and 537-98-4); caffeic acid (3,4-dihydroxycinnamic acid, CAS 331-39-5); (+)-catechin [2-(3,4-dihydroxyphenyl)-3,4-dihydro-2*H*-1-benzopyran, CAS 154-23-4, 16198-00-8, 321-01-7, 4211-28-3, 5323-80-8]; (−)-epicatechin [2-(3,4-dihydroxyphenyl)-3,4-dihydro-2*H*-1-benzopyran-3,5,7-triol, CAS 490-46-0); rutin [3-((6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl)oxy)-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4*H*-1-benzopyran-4-one, CAS 153-18-4]; quercetin (3,3′,4′,5,7-pentahydroxyflavone, CAS 117-39-5); kaempferol (3,4′,5,7-tetrahydroxyflavone, CAS 520-18-3); myricetin (3,3′,4′,5,5′,7-hexahydroxyflavone, CAS 529-44-2); cyanidin (3,3′,4′,5,7-pentahydroxyflavylium, CAS 13306-05-3); delphinidin (3,3′,4′,5,5′,7-hexahydroxyflavylium, CAS 13270-61-6); (+)-gallocatechin [3,4-dihydro-2-(3,4,5-trihydroxyphenyl)-2*H*-1-benzopyran-3,5,7-triol, CAS 970-73-0]; and (−)-epigallocatechin [3,4-dihydro-2-(3,4,5-trihydroxyphenyl)-2*H*-1-benzopyran-3,5,7-triol, CAS 970-74-1].

ACKNOWLEDGMENTS

This study was financially supported by the European Social Fund (EU project 980544) and the Academy of Finland (project 40800). We want to express our gratitude to the Berry Know-How Centre of Inner Savo (Suonenjoki, Finland), Alahovi's Berry Farm (Kuopio, Finland), and the Research Garden of the University of Kuopio for providing the berry samples, and to Ms. Johanna Hulkko for skillful technical assistance.

ABBREVIATIONS

CAS, Chemical Abstract Service; HCl, hydrochloric acid; HPLC, high-performance liquid chromatography; UV, ultraviolet; sp., species.

REFERENCES

1. Arts ICW, van de Putte B, and Hollman PCH. Catechin contents of foods commonly consumed in The

Netherlands. 1. Fruits, vegetables, staple foods, and processed foods. *J Agric Food Chem* 48: 1746–1751, 2000.
2. Bartolomé B, Bengoechea ML, Gálvez MC, Pérez-Ilzarbe FJ, Hernández I, Estrella I, and Gómez-Cordovés C. Photodiode array detection for elucidation of the structure of phenolic compounds. *J Chromatogr A* 655: 119–125, 1993.
3. Bate-Smith EC. Plant phenolics in foods. In: *The Pharmacology of Plant Phenolics*, edited by Fairbairn JW. London: Academic Press, 1958, p.148.
4. Beart JE, Lilley TH, and Haslam E. Plant polyphenols—secondary metabolism and chemical defence: some observations. *Phytochemistry* 24: 33–38, 1985.
5. Costantino L, Albasini A, Rastelli G, and Benvenuti S. Activity of polyphenolic crude extracts as scavengers of superoxide radicals and inhibitors of xanthine oxidase. *Planta Med* 58: 342–344, 1992.
6. Deighton N, Brennan R, Finn C, and Davies HV. Antioxidant properties of domesticated and wild *Rubus* species. *J Sci Food Agric* 80: 1307–1313, 2000.
7. Foo LY and Porter LJ. The structure of tannins of some edible fruits. *J Sci Food Agric* 32: 711–716, 1981.
8. Formica JV and Regelson W. Review of the biology of quercetin and related bioflavonoids. *Food Chem Toxicol* 33: 1061–1080, 1995.
9. Goiffon J-P, Brun M, and Bourrier M-J. High-performance liquid chromatography of red fruit anthocyanins. *J Chromatogr* 537: 101–121, 1991.
10. Häkkinen S, Heinonen M, Kärenlampi S, Mykkänen H, Ruuskanen J, and Törrönen R. Screening of selected flavonoids and phenolic acids in 19 berries. *Food Res Int* 32: 345–353, 1999.
11. Häkkinen SH, Kärenlampi SO, Heinonen IM, Mykkänen HM, and Törrönen AR. Content of the flavonols quercetin, myricetin, and kaempferol in 25 edible berries. *J Agric Food Chem* 47: 2274–2279, 1999.
12. Haslam E. Natural polyphenols (vegetable tannins) as drugs: possible modes of action. *J Nat Prod* 59: 205–215, 1996.
13. Heinonen IM, Lehtonen PJ, and Hopia A. Antioxidant activity of berry and fruit wines and liquors. *J Agric Food Chem* 46: 25–31, 1998.
14. Heller W and Forkmann G. Biosynthesis of flavonoids. In: *The Flavonoids. Advances in Research Since 1986*, edited by Harborne JB. London: Chapmann & Hall, 1994, pp. 499–535.
15. Hertog MGL, Hollman PCH, and Katan MB. Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in The Netherlands. *J Agric Food Chem* 40: 2379–2383, 1992.
16. Hong V and Wrolstad RE. Characterization of anthocyanin containing colorants and fruit juices by HPLC/photodiode array detection. *J Agric Food Chem* 38: 698–708, 1990.
17. Iversen CK. Black currant nectar: effect of processing and storage on anthocyanin and ascorbic acid content. *J Food Sci* 64: 37–41, 1999.
18. Justesen U, Knuthsen P, and Leth T. Quantitative analysis of flavonols, flavones, and flavanones in fruits, vegetables and beverages by high-performance liquid chromatography with photo-diode array and mass spectrometric detection. *J Chromatogr A* 799: 101–110, 1998.
19. Kähkönen MP, Hopia AI, Vuorela HJ, Rauha J-P, Pihlaja K, Kujala TS, and Heinonen M. Antioxidant activity of plant extracts containing phenolic compounds. *J Agric Food Chem* 47: 3954–3962, 1999.
20. Kalt W, Forney CF, Martin A, and Prior RL. Antioxidant capacity, vitamin C, phenolics, and anthocyanins after fresh storage of small fruits. *J Agric Food Chem* 47: 4638–4644, 1999.
21. Kühnau J. The flavonoids: a class of semi-essential food components: their role in human nutrition. *World Rev Nutr Diet* 24: 117–191, 1976.
22. Lairon D and Amiot MJ. Flavonoids in food and natural antioxidants in wine. *Curr Opin Lipidol* 10: 23–28, 1999.
23. Macheix J-J, Fleuriet A, and Billot J. *Fruit Phenolics*. Boca Raton, Florida: CRC Press, Inc., 1990.
24. Middleton E Jr, Kandaswami C, and Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacol Rev* 52: 673–751, 2000.
25. Miller NJ and Rice-Evans CA. The relative contributions of ascorbic acid and phenolic antioxidants to the total antioxidant activity of orange and apple fruit juices and black currant drink. *Food Chem* 60: 331–337, 1997.
26. Morton LW, Abu-Amsha Cacetta R, Puddey IB, and Croft KD. Chemistry and biological effects of dietary phenolic compounds: relevance to cardiovascular disease. *Clin Exp Pharmacol Physiol* 27: 152–159, 2000.
27. Øydvin J. Inheritance of four cyanidin-3-glycosides in red currant. *Hort Res* 14: 1–7, 1974.
28. Packer L, Saliou C, Droy-Lefaix M-T, and Christen Y. Ginkgo biloba extract Egb761: biological actions, antioxidant activity, and regulation of nitric oxide synthase. In: *Flavonoids in Health and Disease*, edited by Rice-Evans C and Packer L. New York, NY: Marcel Dekker, Inc., 1998, pp. 303–341.
29. Parr AJ and Bolwell GP. Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. *J Sci Food Agric* 80: 985–1012, 2000.
30. Porter LJ, Hrstich LN, and Chan BG. The conversion of procyanidins and prodelphidins to cyanidin and delphinidin. *Phytochemistry* 25: 223–230, 1986.
31. Price KR, Casuscelli F, Colquhoun IJ, and Rhodes MJC. Hydroxycinnamic acid esters from broccoli florets. *Phytochemistry* 45: 1683–1687, 1997.
32. Prior RL, Cao G, Martin A, Sofic E, McEwen J, O'Brien C, Lischner N, Ehlenfeldt M, Kalt W, Krewer G, and Mainland CM. Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of *Vaccinium* species. *J Agric Food Chem* 46: 2686–2693, 1998.
33. Rice-Evans CA, Miller NJ, and Paganga G. Struc-

ture–antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic Biol Med* 20: 933–956, 1996.
34. Santos-Buelga C and Scalbert A. Proanthocyanidins and tannin-like compounds—nature, occurrence, dietary intake and effects on nutrition and health. *J Sci Food Agric* 80: 1094–1117, 2000.
35. Schuster B and Herrmann K. Hydroxybenzoic and hydroxycinnamic acid derivatives in soft fruits. *Phytochemistry* 24: 2761–2764, 1985.
36. Shahidi F and Naczk M. *Food Phenolics. Sources, Chemistry, Effects, Applications.* Lancaster, PA: Technomic Publishing Co, Inc., 1995.
37. Stöhr H and Herrmann K. The phenolics of fruits VI. The phenolics of currants, gooseberries and blueberries. Changes in phenolic acids and catechins during development of black currants. *Z Lebensm Unters Forsch* 159: 31–37, 1975.
38. Sun B, Leandro C, Ricardo da Silva JM, and Spranger I. Separation of grape and wine proanthocyanidins according to their degree of polymerization. *J Agric Food Chem* 46: 1390–1396, 1998.
39. Wang SY and Lin H-S. Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. *J Agric Food Chem* 48: 3183–3189, 2000.

Address reprint requests to:
Kaisu Määttä
Institute of Applied Biotechnology
University of Kuopio
P.O. Box 1627
FIN-70211 Kuopio, Finland

E-mail: Kaisu.Maatta@uku.fi

Received for publication October 3, 2000; accepted April 24, 2001.

This article has been cited by:

1. Maja Mikulic-Petkovsek, Ana Slatnar, Franci Stampar, Robert Veberic. 2012. HPLC–MSn identification and quantification of flavonol glycosides in 28 wild and cultivated berry species. *Food Chemistry* **135**:4, 2138-2146. [CrossRef]
2. Flaubert Mbeunkui, Mary H. Grace, Gad G. Yousef, Mary Ann Lila. 2012. Isolation and characterization of flavonols from blackcurrant by high-performance counter-current chromatography and electrospray ionization tandem mass spectrometry. *Journal of Separation Science* **35**:13, 1682-1689. [CrossRef]
3. Ashwin Gopalan, Sharon C. Reuben, Shamima Ahmed, Altaf S. Darvesh, Judit Hohmann, Anupam Bishayee. 2012. The health benefits of blackcurrants. *Food & Function* **3**:8, 795. [CrossRef]
4. Suri Roowi, Alan Crozier. 2011. Flavonoids in Tropical Citrus Species. *Journal of Agricultural and Food Chemistry* 111019152150000. [CrossRef]
5. A.S. Sivam, D. Sun-Waterhouse, C.O. Perera, G.I.N. Waterhouse. 2011. Exploring the interactions between blackcurrant polyphenols, pectin and wheat biopolymers in model breads; a FTIR and HPLC investigation. *Food Chemistry* . [CrossRef]
6. Oskar Laaksonen, Mari Sandell, Emilia Nordlund, Raija-Liisa Heiniö, Hanna-Liisa Malinen, Mari Jaakkola, Heikki Kallio. 2011. The effect of enzymatic treatment on blackcurrant (Ribes nigrum) juice flavour and its stability. *Food Chemistry* . [CrossRef]
7. Oskar Laaksonen, Mari Sandell, Riikka Järvinen, Heikki Kallio. 2011. Orosensory contributing compounds in crowberry (Empetrum nigrum) press-byproducts. *Food Chemistry* **124**:4, 1514-1524. [CrossRef]
8. Kaisu Riihinen, Anu Ryynänen, Marko Toivanen, Eija Könönen, Riitta Törrönen, Carina Tikkanen-Kaukanen. 2011. Antiaggregation potential of berry fractions against pairs of Streptococcus mutans with Fusobacterium nucleatum or Actinomyces naeslundii. *Phytotherapy Research* **25**:1, 81-87. [CrossRef]
9. Kaisa Linderborg, Oskar Laaksonen, Heikki Kallio, Baoru Yang. 2010. Flavonoids, sugars and fruit acids of alpine bearberry (Arctostaphylos alpina) from Finnish Lapland. *Food Research International* . [CrossRef]
10. Oskar Laaksonen, Mari Sandell, Heikki Kallio. 2010. Chemical factors contributing to orosensory profiles of bilberry (Vaccinium myrtillus) fractions. *European Food Research and Technology* **231**:2, 271-285. [CrossRef]
11. Nora Pap, Mika Mahosenaho, Eva Pongrácz, Helmi Mikkonen, Mari Jaakkola, Vesa Virtanen, Liisa Myllykoski, Zsuzsanna Horváth-Hovorka, Cecília Hodúr, Gyula Vatai, Riitta L. Keiski. 2010. Effect of Ultrafiltration on Anthocyanin and Flavonol Content of Black Currant Juice (Ribes nigrum L.). *Food and Bioprocess Technology* . [CrossRef]
12. Soon Yew Tang, Barry Halliwell. 2010. Medicinal plants and antioxidants: What do we learn from cell culture and Caenorhabditis elegans studies?. *Biochemical and Biophysical Research Communications* **394**:1, 1-5. [CrossRef]
13. Janne J. Koskimäki, Juho Hokkanen, Laura Jaakola, Marja Suorsa, Ari Tolonen, Sampo Mattila, Anna Maria Pirttilä, Anja Hohtola. 2009. Flavonoid biosynthesis and degradation play a role in early defence responses of bilberry (Vaccinium myrtillus) against biotic stress. *European Journal of Plant Pathology* **125**:4, 629-640. [CrossRef]
14. Jie Zheng, Heikki Kallio, Baoru Yang. 2009. Effects of latitude and weather conditions on sugars, fruit acids and ascorbic acid in currant (Ribes sp.) cultivars. *Journal of the Science of Food and Agriculture* **89**:12, 2011-2023. [CrossRef]
15. Joerg Gruenwald. 2009. Novel botanical ingredients for beverages. *Clinics in Dermatology* **27**:2, 210-216. [CrossRef]

16. K RIIHINEN, L JAAKOLA, S KARENLAMPI, A HOHTOLA. 2008. Organ-specific distribution of phenolic compounds in bilberry (Vaccinium myrtillus) and 'northblue' blueberry (Vaccinium corymbosum x V. angustifolium). *Food Chemistry* **110**:1, 156-160. [CrossRef]
17. L. Jaakola, J. J. Koskimäki, K. R. Riihinen, A. Tolvanen, A. Hohtola. 2008. Effect of wounding on chalcone synthase and pathogenesis related PR-10 gene expression and content of phenolic compounds in bilberry leaves. *Biologia Plantarum* **52**:2, 391-395. [CrossRef]
18. Courtney L. Meier, Katharine N. Suding, William D. Bowman. 2008. Carbon flux from plants to soil: roots are a below-ground source of phenolic secondary compounds in an alpine ecosystem. *Journal of Ecology* **96**:3, 421-430. [CrossRef]
19. Johanna Rajakangas, Marjo Misikangas, Essi Päivärinta, Marja Mutanen. 2008. Chemoprevention by white currant is mediated by the reduction of nuclear #-catenin and NF-#B levels in Min mice adenomas. *European Journal of Nutrition* **47**:3, 115-122. [CrossRef]
20. Pu Jing, M Monica GiustiNatural pigments of berries **20072431**, 105-146. [CrossRef]
21. Marina Heinonen. 2007. Antioxidant activity and antimicrobial effect of berry phenolics – a Finnish perspective. *Molecular Nutrition & Food Research* **51**:6, 684-691. [CrossRef]
22. J MORILLASRUIZ, J VILLEGASGARCIA, F LOPEZ, M VIDALGUEVARA, P ZAFRILLA. 2006. Effects of polyphenolic antioxidants on exercise-induced oxidative stress. *Clinical Nutrition* **25**:3, 444-453. [CrossRef]
23. Johanna Buchert, Jani M Koponen, Marjaana Suutarinen, Annikka Mustranta, Martina Lille, Riitta Törrönen, Kaisa Poutanen. 2005. Effect of enzyme-aided pressing on anthocyanin yield and profiles in bilberry and blackcurrant juices. *Journal of the Science of Food and Agriculture* **85**:15, 2548-2556. [CrossRef]
24. Riitta Puupponen-Pimi#, Liisa Nohynek, Hanna-Leena Alakomi, Kirsi-Marja Oksman-Caldentey. 2005. Bioactive berry compounds?novel tools against human pathogens. *Applied Microbiology and Biotechnology* **67**:1, 8-18. [CrossRef]
25. Riitta Puupponen-Pimiä, Liisa Nohynek, Hanna-Leena Alakomi, Kirsi-Marja Oksman-Caldentey. 2005. The action of berry phenolics against human intestinal pathogens. *BioFactors* **23**:4, 243-251. [CrossRef]
26. 2003. Trend of Most Cited Papers (2001-2002) in ARS. *Antioxidants & Redox Signaling* **5**:6, 813-815. [Citation] [Full Text PDF] [Full Text PDF with Links]
27. Catherine A. Rice-Evans , Debasis Bagchi . 2001. Nutritional Proanthocyanidins, Flavonoids, and Related Phenols. *Antioxidants & Redox Signaling* **3**:6, 939-940. [Citation] [Full Text PDF] [Full Text PDF with Links]