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Distribution and Contents of Phenolic Compounds in Eighteen Scandinavian Berry Species

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Berries contain a wide range of phenolic compounds in different conjugated forms, a fact that makes their simultaneous analysis a difficult task. In this work, soluble and insoluble phenolic compounds were identified and quantified in 18 species of berries by reversed phase high-performance liquid chromatography combined with diode array detection. The analytical results and literature data were used for the identification of the predominant conjugated hydroxycinnamic acids, flavonol glycosides, and anthocyanins in berries from six families, viz. Grossulariaceae, Ericaceae, Rosaceae, Empetraceae, Elaeagnaceae, and Caprifoliaceae. The study showed distinctive similarities among berry species of the same family in the distribution of conjugated forms of phenolic compounds but differences in chromatographic profiles of conjugates and compositions of aglycones especially in the case of anthocyanins. The chromatographic profiles of chokeberry and the related sweet rowanberry (Rosaceae) were exceptionally similar. These data are informative to studies on the authenticity of berry raw materials as well as to those on the evaluation of berries as sources of phenolic compounds.

KEYWORDS: Berries; phenolic compounds; HPLC; diode array detection

INTRODUCTION

Fruits and berries contain phenolic acids and flavonoids (**Figure 1**). The phenolic compounds are combined with sugars or other polyols via O-glycosidic bonds (flavonols, anthocyanidins, and hydroxycinnamic acids) or ester bonds (hydroxycinnamic acids) (*1*). The distribution of these conjugated forms of phenolic compounds is typical for the plant species, which might be utilized to study the authenticity of the raw material in manufactured jams, jellies, juices, and wines (*1*). The contents of phenolic compounds in plant foods have also received much attention during recent years because of their biological properties imparting possible benefits to human health (*2*−*5*). In consideration of berries as a source of phenolic compounds as well as in the characterization of the composition of individual conjugates, a simultaneous determination of all major phenolic classes is needed.

Berries are known as rich sources of hydroxycinnamic acids, ellagitannins, flavonol glycosides, anthocyanins, flavan-3-ols, and proanthocyanidins (*1*, *6*). Ellagitannins, which are abundant in some species of the Rosaceae family (raspberry, arctic bramble, cloudberry, and strawberry) (*7*), were not investigated

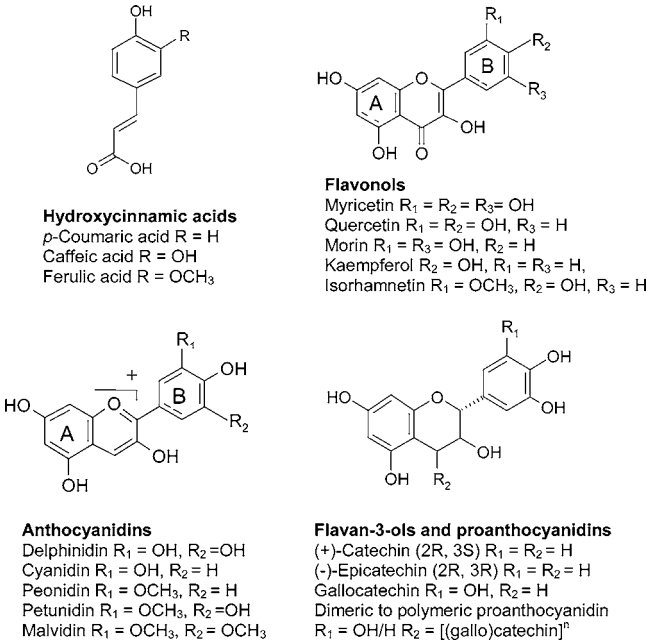

Figure 1. Structures of aglycones of phenolic compounds.

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Table 1. Description of the Berries Studied

Latin name	trivial name and cultivar (cv.)	color, content[a] of soluble solids, and % moisture
	family Grossulariaceae	
Ribes nigrum L.	black currant, cv. Öjebyn	shiny black, 16.2 °Brix, 78%
Ribes nigrum L.	green currant, cv. unnamed	yellowish-green, 15.9 °Brix, 78%
Ribes × *pallidum* Otto and F. Dietr	red currant, cv. Red Dutch	shiny scarlet, 9.7 °Brix, 84%
Ribes × *pallidum* Otto and F. Dietr	white currant, cv. White Dutch	shiny white, 13.0 °Brix, 81%
Ribes uva-crispa L.	gooseberry, red, cv. Hinnonmaki's red	deep red, 12.0 °Brix, 84%
Ribes uva-crispa L.	gooseberry, yellow, cv. Hinnonmaki's yellow	yellow, 9.3 °Brix, 87%
	family Ericaceae	
Vaccinium uliginosum L.	bog whortleberry (or northern bilberry), wild	blue, 10.0–11.9 °Brix, 86–88%
Vaccinium myrtillus L.	bilberry (or whortleberry), wild	bluish-black, 9.5 °Brix, 86%
Vaccinium corymbosum L.	half-high blueberry, cv. Aino	blue, 13.2 °Brix, 84%
Vaccinium vitis-idaea L.	lingonberry (or cowberry), wild	scarlet, 12.4 °Brix, 84%
Vaccinium oxycoccos L.	cranberry, wild	dark red, 9.8 °Brix, 86%
	family Rosaceae	
Aronia mitschurinii (or Aronia melanocarpa var. grandifolia)	chokeberry, cv. Viking	reddish-black, 19.1 °Brix, 77%
x Crataegosorbus mitschurinii[b]	sweet rowanberry, cv. Granatnaja	red, 16.6 °Brix, 82%
Prunus spinosa L.	blackthorn, wild	bluish-black, 19.1 °Brix, 68%
	family Empetraceae	
Empetrum hermaphroditum L.	northern crowberry, wild	shiny black, 7.1 °Brix, 89%
Empetrum nigrum L.	southern crowberry, wild	shiny black, 5.4 °Brix, 92%
	family Elaeagnaceae	
Hippóphaë rhamnoides L.	sea buckthorn, cv. mixture[c]	orange, 8.9 °Brix, 83%
	family Caprifoliaceae	
Sambucus nigra L.	elderberry, cv. unknown	shiny black, 12.2 °Brix, 82%

[a] Soluble solids as °Brix and moisture contents are means of triplicate analyses from the edible part of the fresh berry. [b] Crossbreed of *Crataegus sanguinea* Pall. and *Sorbus aucuparia* L. [c] Mixture of cultivars Obilnaja, Zheltaja Rannaja, Kaliningradskaja, Finskaja, and Vorobjovskaja.

in the present study. As a general rule for contents, the strongly colored berries, black currant, bilberry, blueberry, bog whortleberry, crowberry, chokeberry, and elderberry, contain high levels of anthocyanins as pigments as well as flavonol glycosides and/or conjugated hydroxycinnamic acids (*1*, *6*, *8*). Previously, the distribution of anthocyanins has been studied in black and red currant (*9*), bilberry (*10*), blueberry (*11*), lingonberry (*12*), cranberry (*13*), chokeberry (*14*), and elderberry (*15*), and there is also some scattered data on other berries (*8*, *12*−*14*, *16*). Fewer studies are published on the distribution of flavonol glycosides and conjugated hydroxycinnamic acids in berries (*9*, *14*, *17*−*21*). Flavan-3-ols and low molecular weight (LMW) proanthocyanidins have been studied in some common berries among other plant foods (*22*−*24*). The composition of structural units in high molecular weight (HMW) proanthocyanidins has been characterized (*25*, *26*), and there is one very recent study on their content in the most common berries (*24*).

The isolation of structurally diverse phenolic classes from the sample matrix is demanding with respect to their solubility and stability characteristics (*27*). Previously, we presented a sample preparation technique based on sequential extraction (*9*, *28*). Phenolic compounds were extracted mainly with ethyl acetate, but pH-dependent flavylium cations of anthocyanins were extracted subsequently to acidified methanol, and insoluble HMW proanthocyanidins were converted to anthocyanidins by acid hydrolysis of the extraction residue (*9*, *28*). In the present study, a liquid−liquid extraction step was added to the procedure for eliminating coeluting phenolic acids interfering with the analysis of flavan-3-ols and LMW proanthocyanidins. This study presents and compares the distribution and contents of the free and conjugated forms of hydroxycinnamic acids, flavonols, anthocyanidins as well as flavan-3-ols, and LMW and insoluble proanthocyanidins in the diverse Finnish and Swedish berries (**Table 1**). Earlier literature data enabled further identification of the conjugated forms of phenolic compounds in the studied berries.

MATERIALS AND METHODS

Berry Samples. Ten species of cultivated berries and eight species of wild berries were studied (**Table 1**). The berries were harvested at maturity in eastern Finland at 62−63° N (Kuopio, Leppävaara, Rautavaara, Savonlinna), northeastern Finland at 66° N (Kuusamo), northern Finland at 69° N (Ivalojoki), and eastern Sweden at 60° N (Uppsala) in the year 2002. The cultivated berries were obtained from the Research Garden of the University of Kuopio, except yellow gooseberry, green currant, and blueberry, which were purchased from local farmers. The berries were frozen at −24 ± 2 °C until analyzed within 3 months (Finnish berries) or within 6 months (Swedish berries). The samples of homogenized berries were freeze-dried overnight for the analysis of the moisture content and centrifuged for the separation of juice in the analysis of the soluble solids as °Brix. A portion of raspberry (a mixture of cultivars) was freeze-dried for the analysis with the present and alternative methods (*20*, *22*, *29*).

Standards. 4-Hydroxybenzoic acid, chlorogenic acid, *p*-coumaric acid, caffeic acid, ferulic acid, sinapic acid, rutin (quercetin 3-rhamnosylglucoside), quercetin, kaempferol, morin, (+)-catechin, and (−)-epicatechin were purchased from Sigma Chemical Co. (St. Louis, MO). Isorhamnetin and myricetin were obtained from Fluka (Buchs, Switzerland). Cyanidin and delphinidin chlorides were purchased from Extrasynthese (Geney Cedex, France). These commercial standards were dissolved in methanol to a concentration of ∼1 mg/mL for the preparation of calibration curves. A mixture of 3-glucosides of delphinidin, cyanidin, petunidin, pelargonidin, peonidin, and malvidin (5 μmol of each) was obtained from Polyphenols AS (Sandnes, Norway) and dissolved in 20 mL of methanol for the preparation of calibration curves.

Sample Extraction and Purification. The extraction procedure for soluble and insoluble phenolic compounds, presented in our previous study (*28*), was modified for the analysis of flavan-3-ols and LMW proanthocyanidins. The edible parts of frozen berries were homogenized,

and the samples (5 g) were weighed in centrifuge tubes for extraction. Morin (0.1 mL; 1 mg/mL methanol) was added as an internal standard as it showed a similar solubility as flavan-3-ols. The extractions were performed by repeated vigorous vortexing of samples with ethyl acetate (4 × 10 mL) and intermittent centrifugation. The combined ethyl acetate extracts containing conjugated hydroxycinnamic acids, morin, flavonol glycosides, flavan-3-ols, and LMW proanthocyanidins were divided into two portions (20 mL each).

One portion was evaporated to dryness using a rotary evaporator and dissolved in 1 mL of methanol. The portion was subjected to direct high-performance liquid chromatography (HPLC) analysis of the native free and conjugated forms of hydroxycinnamic acids and flavonols. Afterward, this part of the extract was acidified to 0.6 M with concentrated HCl and heated for 5 min in a boiling water bath (70−80 °C) to release the aglycones from flavonol *O*-glycosides for analysis by HPLC (*28*).

Because the conjugated hydroxycinnamic acids coeluted with peaks of favan-3-ols and LMW proanthocyanidins in our previous studies on currants, here, we tried to separate these phenolic compounds by liquid−liquid extraction. The second portion of the ethyl acetate extract was extracted with sodium acetate buffer (2 × 10 mL, 0.1 M, pH 7.0) and then with 10 mL of water to remove ionizable phenolic acids into the water phase. After this purification step, the ethyl acetate phase was evaporated to dryness in a rotary evaporator and dissolved in 1 mL of methanol for analysis of flavan-3-ols and LMW proanthocyanidins by HPLC.

After the ethyl acetate extraction, the berry residue was acidified with HCl (2 M, 2 mL) and extracted with methanol four times to a total extraction volume of ∼50 mL mainly to obtain anthocyanins as flavylium cations but also residues of above compounds not extracted in ethyl acetate. An aliquot of the methanol extract (10 mL) was evaporated to dryness in a rotary evaporator, dissolved in 1 mL of methanol, and analyzed by HPLC (anthocyanins in the methanol extracts of strongly pigmented berries were analyzed without concentration). The berry residue after this extraction was suspended in 10 mL of methanol, acidified to 0.6 M with concentrated HCl, and refluxed for 2 h (60−70 °C) for the conversion of insoluble HMW proanthocyanidins to anthocyanidins and for the release of insoluble glycosidic forms of hydroxycinnamic acids and flavonols. This acid-hydrolyzed berry residue was subjected to direct HPLC analysis.

Analysis of Hydroxycinnamic Acids, Flavonols, Flavan-3-ols, and LMW Proanthocyanidins with Alternative Methods. To test the validity of the method presented above for the quantitative analysis of hydroxycinnamic acids, flavonols, flavan-3-ols, and LMW proanthocyanidins, the method was compared with the alternative methods using a freeze-dried raspberry sample. In the present method, powdered freeze-dried raspberry (1 g) was solubilized in water (4 g) and was prepared as described above for frozen samples. Flavonol glycosides were analyzed as aglycones using the methanolic acid hydrolysis method described by Häkkinen and Auriola (*20*). Phenolic acids were analyzed by the method of Mattila and Kumpulainen (*29*). In this method, free hydroxycinnamic acids (including chlorogenic acid) were extracted with a mixture of methanol and 10% acetic acid while total phenolic acids (free + bound forms) were extracted using diethyl ether/ethyl acetate (1:1) after the first alkaline and then acid hydrolysis (during this analysis chlorogenic acid was deconjugated to caffeic acid). Flavan-3-ols and LMW proanthocyanidins were extracted with 75% aqueous methanol and analyzed with a postcolumn derivatization reaction with *p*-dimethylaminocinnamaldehyde as described previously (*30*, *31*). Chromatographic analyses, identifications, and quantification of alternative methods were performed for flavonol aglycones as described in the present study and for hydroxycinnamic acids (*29*), flavan-3-ols, and LMW proanthocyanidins (*30*, *31*) as described previously.

Chromatographic Analyses. Extracts as well as acid hydrolyzates of extracts and berry residues were filtered through a 0.45 μm syringe filter before injection into HPLC. HPLC separation of the phenolic compounds was achieved on a 125 mm × 3 mm i.d., 5 μm, LiChroCART Purospher RP-18e column (Merck, Darmstadt, Germany) protected with a guard column of the same material (4 mm × 4 mm). A 20 min linear gradient of acetonitrile in 1% formic acid was used to separate the free and conjugated forms of hydroxycinnamic acids and flavonols as well as anthocyanidins. On the other hand, a step gradient of acetonitrile in 5% formic acid was used to separate anthocyanins in extracts as follows: 5−10% acetonitrile (0−5 min), 10% acetonitrile (5−10 min), 10−40% acetonitrile (10−25 min), and finally 40−90% acetonitrile (25−35 min) (*28*). For a better separation of flavan-3-ols and dimeric and trimeric proanthocyanidins in the purified ethyl acetate extracts, a new gradient of acetonitrile in 5% formic acid was developed. In the beginning, 5% acetonitrile was used isocratically (0−5 min) and then increased to 20% in a linear gradient (5−35 min). All three gradients were at a flow rate of 0.5 mL/min and followed by raising acetonitrile to 90% in 10 min, a return to initial conditions in 5 min, and then reequilibration of the column in 10 min. The chromatographic performance was followed by analysis of a standard mixture (4-hydroxybenzoic acid, caffeic acid, and rutin) in the beginning of every sample series.

Identification and Quantification. HPLC combined with diode array detection was used for UV/vis spectroscopic analysis and quantification. The identification of the free and conjugated forms of phenolic compounds in the chromatograms was based on the retention times and on the comparison of the shapes of their UV/vis spectra with those of the representative standards and our earlier data on conjugates (*9*, *28*). LMW proanthocyanidins showed similar UV spectra to flavan-3-ols (*32*). Further identification was based on literature data on known conjugated hydroxycinnamic acids (*9*, *17*−*19*), flavonol glycosides (*9*, *14*, *17*, *18*, *20*, *21*), and anthocyanins (*8*−*10*, *12*−*16*) in respective berries. The identification of flavonol glycosides was confirmed by releasing the aglycones by acid hydrolysis of ethyl acetate extracts. The polymer of insoluble HMW proanthocyanidins was defined by the ratio of procyanidin and prodelphinidin, which originate from the monomeric units of (+)-catechin/(−)-epicatechin and (+)-gallocatechin/(−)-epigallocatechin in the structure, respectively (**Figure 1**). Upon acid hydrolysis, the conversion of proanthocyanidins to cyanidin and delphinidin has been shown to occur by oxidation following acid-catalyzed cleavage of the interflavanoid bond (*33*).

All identified individual compounds were quantified using the standard curves of representative standards, for which the response factors were from twice-prepared fresh solutions in the following concentration ranges of aglycones: anthocyanins (1.5−85 mg/L) and other phenolic compounds (2−250 mg/L). The response factors of anthocyanidins and anthocyanins were determined in acidified methanol (0.6 M HCl). The quantified contents were given for the weight of the phenolic unit of the molecule (aglycone) using the response factors of available standard glycosides (rutin and anthocyanidin 3-glucosides) or aglycones (hydroxycinnamic acids, flavonols, flavan-3-ols, and anthocyanidins) near their wavelengths of maximum absorption as previously reported (*9*). LMW proanthocyanidins were quantified using the response factor of (−)-epicatechin. Morin was used as an internal standard in the quantification of flavan-3-ols and LMW proanthocyanidins to correct losses due to the purification step of the ethyl acetate extract. The contents of phenolic compounds were quantified, as total aglycones, from the sum of the soluble forms in the ethyl acetate and methanol extracts and after acid hydrolysis of the extraction residue.

RESULTS AND DISCUSSION

Analysis of Soluble and Insoluble Phenolic Compounds. The challenge in the isolation of diverse phenolic classes from a sample matrix is attributable to their different solubility and partitioning in the extraction solvents and/or the possible formation of artifacts during the extraction process (*27*). Here, we present a modified stepwise extraction procedure to, as much as possible, avoid these problems in the analysis of soluble and insoluble phenolic compounds in berries. First, the major parts of easily degradable free and conjugated forms of hydroxycinnamic acids and flavonols as well as flavan-3-ols and LMW proanthocyanidins were extracted from the homogenized frozen berry in ethyl acetate. Subsequently, the residue was acidified with hydrochloric acid to favor extractability and stability of anthocyanins as flavylium cations in methanol. Moreover, the extraction residue from methanol extraction was acid hydrolyzed

to liberate residues of glycosylated forms and/or cell wall-bound flavonols and hydroxycinnamic acids as aglycones and insoluble HMW proanthocyanidins as anthocyanidins. In our previous studies (*9*, *28*), conjugated hydroxycinnamic acids coeluted with the peaks of flavan-3-ols and LMW proanthocyanidins in ethyl acetate extracts of black, green, red, and white currants. Therefore, liquid−liquid extraction of one-half of the ethyl acetate extract with sodium acetate buffer was added to the experimental procedure in a trial to solve this problem by elimination of phenolic acids before the analysis of flavan-3-ols and LMW proanthocyanidins.

The extractability of phenolic compounds in solvents was evaluated by their quantification in the ethyl acetate extract, in the subsequent methanol extract, and in the extraction residue. The free and conjugated forms of hydroxycinnamic acids and flavonols were extracted mainly in ethyl acetate (91± 8% of total content, $n = 22$). The exceptions were black and green currants, gooseberries, bog whortleberry, chokeberry, sweet rowanberry, blackthorn, sea buckthorn, and elderberry, in which the excess of flavonol glycosides (24−65% of total content) was subsequently extracted in methanol. These berries contain high contents and/or diglycosides of flavonols that are more polar and less soluble in ethyl acetate than monoglycosides. The residues of hydroxycinnamic acids (28−51% of total content) in the methanol extracts of chokeberry, sweet rowanberry, and blackthorn were caused by their high contents. Negligible amounts (0−3%) of hydroxycinnamic acids and flavonols were released in acid hydrolysis of extraction residues. Exceptions were hydroxycinnamic acids in the extraction residue of southern crowberry and elderberry (24 and 19% of total content, respectively) and flavonols in the extraction residue of sea buckthorn (31% of total content), which were assumed to represent cell wall-bound phenolic compounds rather than residues of the extractable forms. Anthocyanins were mainly extracted in methanol (98 ± 1% of total content, $n = 14$), and the observed high preextracted amounts of anthocyanins in the ethyl acetate extracts of blueberry and red gooseberry (9 and 33% of total content, respectively) were due to less polar acylated forms. The recoveries were not determined since adding available standards to the berry samples would poorly represent recoveries of the variable conjugated forms of phenolic compounds. The sum of the contents in the ethyl acetate extract, the methanol extract, and the berry residue was considered to provide the best available result of the quantities of soluble and insoluble phenolic compounds in berries. The results are expressed on the weight of aglycone, since the weight of sugar varies in the conjugates and aglycones were released in the acid hydrolysis of the extraction residues (**Table 2**). Because the HMW proanthocyanidins were semiquantitatively converted to cyanidin and delphinidin (*33*, *34*), their distribution is expressed as a ratio of procyanidin and prodelphinidin and contents as levels in **Table 2**.

Freeze-dried raspberry was analyzed with this method and with alternative methods, where the basis of sample pretreatment and quantification was different. The content of *p*-coumaric acid was higher (117 vs 80 mg/kg), and the sums of caffeic, ferulic, and sinapic acids were lower (53 vs 101 mg/kg) when analyzed with the present method as compared to an alternative method, wherein aglycones were released by acid and alkali hydrolysis (*29*). The difference may be due to chlorogenic acid (t_R 9.9 min), which was found to coelute with one predominant *p*-coumaric acid ester (t_R 10.0 min) in the present chromatographic separations of conjugates. The analysis of extracted flavonol glycosides by the present method gave higher contents (42 vs 16 mg/kg) than the quantification of their aglycones after acid hydrolysis with the alternative method (*20*). Previously, the contents of flavonols were corrected with recoveries to balance these losses in acid hydrolysis (*35*). According to a comparison to the alternative method, the present method underestimates the contents of flavan-3-ols (106 vs 505 mg/kg) and LMW proanthocyanidins (125 vs 1488 mg/kg). This difference may be due to the lower extractability of flavan-3-ols and LMW proanthocyanidins from the homogenized berry in ethyl acetate than in aqueous methanol used in the alternative method. After ethyl acetate extraction, the possible residues of these two phenolic classes were not quantified in the subsequent methanol extracts because of the overlapping peaks of anthocyanins and/or residual conjugated hydroxycinnamic acids (chromatograms not shown). However, the contents of flavan-3-ols in 18 berry species obtained using this method are shown in **Table 2** for comparison of their distribution and relative contents. Because LMW proanthocyanidins had an even lower extractability in ethyl acetate than flavan-3-ols (*34*), their contents are only expressed as levels for the mutual comparison in **Table 2**.

Phenolic Compounds in Berries of Grossulariaceae Family. The conjugated forms of phenolic compounds in the currants of the Grossulariaceae family were identified and presented in our previous study (*9*). Caffeoyl- and *p*-coumaroylglucosides were the typical conjugated hydroxycinnamic acids in this family. In this study, *p*-coumaric acid dominated in the other berries except yellow gooseberry (**Table 2**). These contents and distributions of hydroxycinnamic acids, presented in **Table 2**, are comparable to earlier studies (*9*, *19*, *36*).

In currants, the predominant flavonols were conjugated typically with rutinose, glucose, and malonylglucose (*9*). The composition of flavonol glycosides in gooseberries resembled that of black currant (**Figure 2**) and other currants (*9*, *28*), but their deconjugation to the aglycones by acid hydrolysis revealed an unexpected occurrence of isorhamnetin (60−68%) besides quercetin (32−40%). Therefore, the late-eluting predominant flavonol glycoside (t_R 17.7 min) in gooseberries was identified as isorhamnetin glycoside and quantified as isorhamnetin (**Figure 2** and **Table 2**). There was no supportive identification in the literature, but it is possible that isorhamnetin aglycone in gooseberries was previously identified as kaempferol with a closely related UV/vis spectrum (absorption maxima at 254 and 371 nm for isorhamnetin and at 265 and 367 nm for kaempferol) and retention time in the used gradient elution on a reverse phase column (*35*). Myricetin was the major aglycone in black currant as in the study of Häkkinen et al. (*35*) but inconsistent with our previous results (*9*) (**Table 2**).

The composition of anthocyanins showed that the predominant sugars (glucose and rutinose) were combined with both cyanidin and delphinidin in black currant but only with cyanidin in red gooseberry (*9*, *37*, *38*) (**Figure 2**). The higher content of delphinidin as compared to cyanidin in black currant is inconsistent with our previous results but is in agreement with those reported by Iversen (*39*) (**Table 2**). This pronounced content of delphinidin and myricetin in black currant, both having trihydroxylation at the B ring (**Figure 1**), might result from the same environmental or maturity factors. Red currant (cv. Red Dutch) was distinguished by its composition of six cyanidin glycosides including 3-glucoside, three diglycosides, and two triglycosides (2^G-glucosylrutinoside and 2^G-xylosylrutinoside) (*40*).

Table 2. Contents[a] and Levels[b] of Phenolic Compounds[c] in Berries of the Families Grossulariaceae, Ericaceae, Rosaceae, Empetraceae, Elaeagnaceae, and Caprifoliaceae

	HCA (conjugates)		flavonols (glycosides)				anthocyanidins (anthocyanins)						flavan-3-ols[d]		levels of proanthocyanidins	
berry and origin	*p*-C	C/F	Myr	Quer	Kaemp	Isorham	Dp	Cy	Pet	Peo	Mv	unknown[e]	(+)-C	(–)-EC	LMW	HMW, PC:PD
							Grossulariaceae									
black currant (Kuopio)	39	27	93	50	13	ND	2440	1452	ND	ND	ND	225	8	11	*	***, 43:57
green currant (Uppsala)	60	31	8	33	12	ND	ND	ND	ND	ND	ND	ND	6	3	ND	***, 38:62
red currant (Kuopio)	11	5	tr	4	tr	ND	ND	213	ND	ND	ND	ND	5	2	ND	*, 13:87
white currant (Kuopio)	31	10	1	12	tr	ND	ND	ND	ND	ND	ND	ND	19	ND	ND	*, 7:93
gooseberry, red (Kuopio)	27	16	tr	14	tr	18	ND	240	ND	ND	ND	ND	7	11	ND	*, 42:58
gooseberry, yellow (Kuopio)	10	41	tr	13	1	15	ND	ND	ND	ND	ND	ND	4	4	ND	*, 53:47
							Ericaceae									
bog whortleberry (Ivalojoki)	8	3	202	587	ND	ND	730	451	373	52	863	145	ND	19	*	*, 100:0
bog whortleberry (Kuusamo)	11	6	342	681	ND	ND	1661	159	879	NA[g]	1525	94	ND	28	*	*, 100:0
bog whortleberry (Kuopio)	11	6	244	577	ND	ND	1068	130	745	NA	1572	109	ND	20	*	*, 100:0
bilberry (Savonlinna)	65	111	31	81	tr	ND	2758	2933	856	178	1284	71	7	68	*	*, 86:14
blueberry (Kuopio)	11	325	17	95	ND	ND	502	214	196	NA	270	293	6	7	*	*, 88:12
lingonberry (Kuopio)	85	23	ND	131	tr	ND	ND	1286	ND	17	ND	ND	214	42	****	*, 100:0
cranberry (Rautavaara)	11	65	63	207	tr	ND	ND	379	ND	481	ND	ND	24	7	**	**, 72:28
							Rosaceae									
chokeberry (Kuopio)	60	832	ND	348	tr	ND	ND	8421	ND	ND	ND	ND	ND	19	ND	***, 100:0
sweet rowanberry (Kuopio)	34	649	ND	119	tr	ND	ND	881	ND	ND	ND	ND	ND	8	ND	***, 100:0
blackthorn (Uppsala)	25	379	ND	207	ND	ND	ND	225	ND	316	ND	ND	14	ND	ND	****, 93:7
							Empetraceae									
northern crowberry (Ivalojoki)	122	25	76	94	ND	ND	1774	2522	784	984	1544	68	35	89	***	*, 45:55
southern crowberry (Kuusamo)	47	13	28	31	ND	ND	909	966	449	478	1258	28	16	44	*	*, 49:51
							Elaeagnaceae									
sea buckthorn (Kuopio)	tr	tr	84	172	ND	167	ND	ND	ND	ND	ND	ND	4	2	ND	*, 58:42
							Caprifoliaceae									
elderberry (Uppsala)	18	179	ND	331	tr	ND	ND	3316	ND	ND	ND	ND	ND	ND	*	*, 100:0

[a] Contents of duplicate assays are expressed as means in mg/kg of fresh weight for the weight of the aglycone. [b] Levels of LMW: not detected, ND < 1 mg/kg; *, 1–10 mg/kg; **, 15–20 mg/kg; ***, approximately 100 mg/kg; ****, approximately 300 mg/kg. Levels of insoluble HMW: *, 10–50 mg/kg; **, 60–90 mg/kg; ***, approximately 100 mg/kg; ****, approximately 1000 mg/kg. [c] Abbreviations used for phenolic compounds: HCA, hydroxycinnamic acid; *p*-C, *p*-coumaric acid; C/F, caffeic or ferulic acid; Myr, myricetin; Quer, quercetin; Kaemp, kaempferol; Isorham, isorhamnetin; Dp, delphinidin; Cy, cyanidin; Pet, petunidin; Peo, peonidin; Mv, malvidin; (+)-C, (+)-catechin; (–)-EC, (–)-epicatechin; PC, procyanidin; PD, prodelphinidin. [d] Contents of flavan-3-ols are corrected according to the internal standard morin. [e] Unknown anthocyanins were not identified by their UV/vis maximums and retention times. [f] ND, not detected; NA, not analyzed; tr, traces detected as aglycones or conjugates. [g] Peonidin 3-glucoside and 3-arabinoside coeluted with malvidin 3-galactoside and 3-glucoside, respectively, in the applied gradient elution. Therefore, the malvidin content includes partly peonidin in bilberry, blueberry, and bog whortleberries.

The major flavan-3-ols were (+)-catechin in green, red, and white currants and (−)-epicatechin in black currant and red gooseberry. This composition is different from those reported for Dutch currants and gooseberry by Arts et al. (*23*). The rare prodelphinidin units were the predominant structural units of insoluble HMW proanthocyanidins in the four currants and red gooseberry, but procyanidin units dominated in yellow gooseberry in agreement with the literature (*25*, *28*).

Phenolic Compounds in Berries of Ericaceae Family. Berries of the Ericaceae family showed a variable distribution of conjugated hydroxycinnamic acids. In distinct similarity, the dominant chlorogenic acid was responsible for the high contents

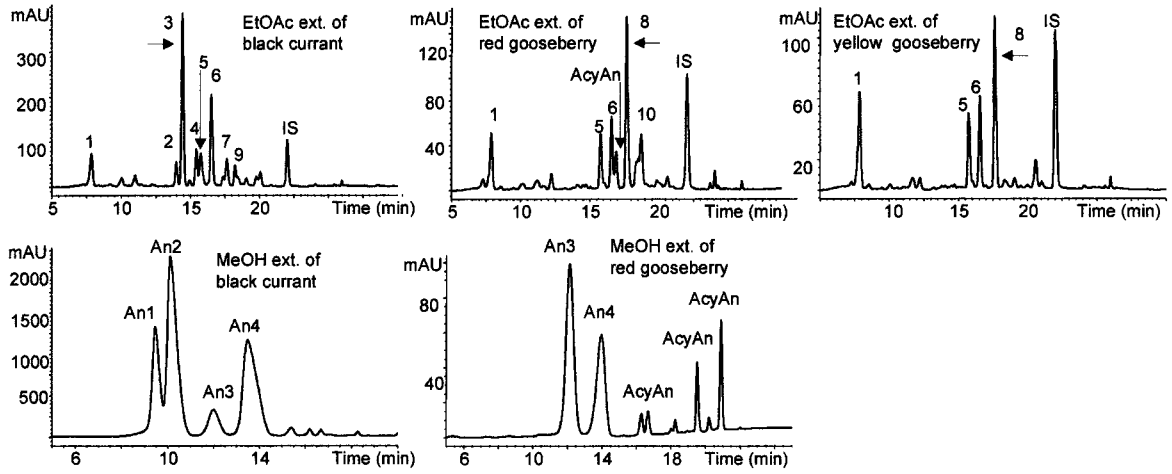

Figure 2. HPLC-DAD chromatograms of black currant and gooseberries of the Grossulariaceae family recorded at 360 nm for the ethyl acetate extracts (EtOAc ext.) and at 520 nm for methanol extracts (MeOH ext.). Peak identification: 1, caffeoyl glucose; 2, myricetin 3-rutinoside; 3, myricetin 3-glucoside; 4, myricetin 3-malonylglucoside; 5, quercetin 3-rutinoside; 6, quercetin 3-glucoside; 7, quercetin 3-malonylglucoside; 8, isorhamnetin glycoside; 9, kaempferol 3-glucoside; 10, *p*-coumaric acid ester; IS, internal standard morin; An1, delphinidin 3-glucoside; An2, delphinidin 3-rutinoside; An3, cyanidin 3-glucoside; An4, cyanidin 3-rutinoside; and AcyAn, acylated anthocyanins.

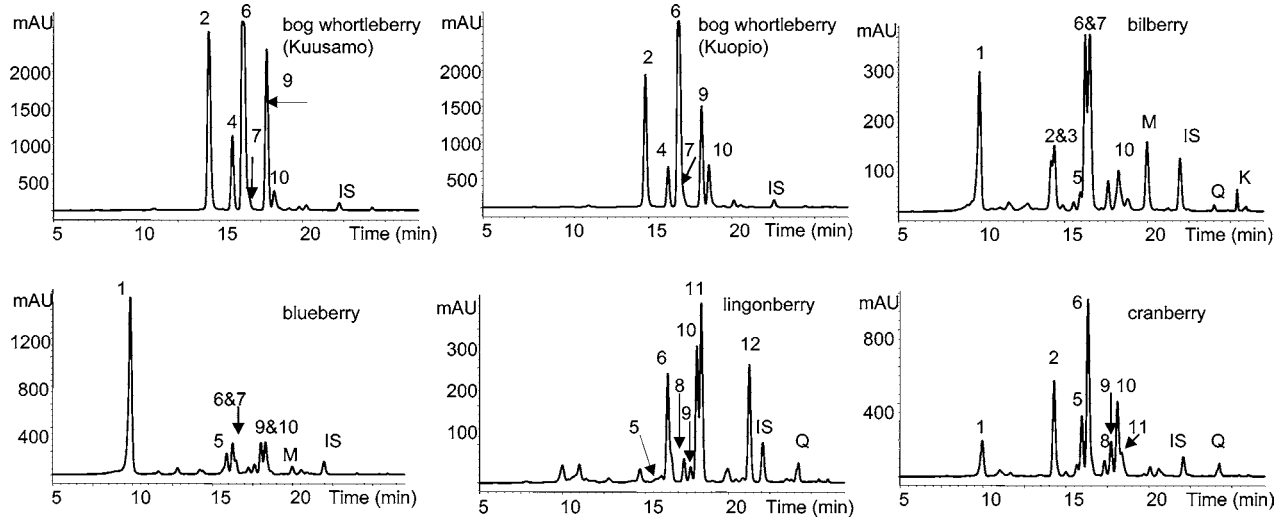

Figure 3. HPLC-DAD chromatograms of ethyl acetate extracts of berries of *Vaccinium* species (family Ericaceae) recorded at 360 nm. Peak identification: 1, chlorogenic acid; 2, myricetin 3-galactoside; 3, myricetin 3-glucoside; 4 and 5, quercetin glycosides; 6, quercetin 3-galactoside; 7, quercetin 3-glucoside; 8, quercetin 3-xyloside; 9, quercetin 3-arabinoside; 10, quercetin 3-rhamnoside; 11 and 12, quercetin glycosides; M, myricetin; Q, quercetin; K, kaempferol; and IS, internal standard morin.

of hydroxycinnamic acids in bilberry, half-high blueberry, and cranberry, in agreement with the literature (*14*, *18*, *19*) (**Figure 3**, **Table 2**). *p*-Coumaric acid was the major hydroxycinnamic acid in lingonberry as reported by Häkkinen et al. (*36*). The content of hydroxycinnamic acids was modest in bog whortleberry as compared to the related berries of *Vaccinium* species (**Table 2**).

Previous identification data (*14*, *18*, *20*, *21*) were utilized in identifying the characteristic flavonol glycosides as myricetin 3-galactoside and quercetin 3-galactoside, 3-glucoside, 3-arabinoside, 3-xyloside, and 3-rhamnoside in berries of *Vaccinium* species. The chromatographic profiles of flavonol glycosides were characteristic for different species in the Ericaceae family (**Figure 3**), but a distinctive similarity was the occurrence of quercetin 3-galactoside and 3-rhamnoside in all berries studied. Two late-eluting flavonol glycosides (t_R 18.4 and 21.3 min) in lingonberry (**Figure 3**) showed a UV/vis maximum at 348 nm typical for kaempferol glycosides (*9*). However, because it was quercetin that was mainly released (97%) after the acid hydrolysis of flavonol glycosides, these compounds were identified as glycosides of quercetin, which usually exhibit a maximum at 354 nm (*9*). The quercetin glycosides with a maximum at 348 nm might have experienced a shift to lower wavelengths due to additional methylation, glycosylation, or acylation of hydroxyl groups (*41*). Previously, kaempferol glycosides were identified in Canadian cultivated lingonberry, cv. Amberland (*14*), but the content of kaempferol has been negligible and not detected in the wild lingonberry according to other studies (*35*, *36*). Quercetin (67−95%) dominated over myricetin (15−33%) in the berries of the Ericaceae family except lingonberry, where no myricetin was detected in accordance with the literature (*35*, *42*) (**Table 2**).

The chromatographic profiles of anthocyanins are different among bilberry, blueberry, lingonberry, and cranberry (*10*−*14*). Furthermore, according to the present results, the compositions of anthocyanidins (aglycones of anthocyanins) were distinguishable to differentiate the species of berries in the Ericaceae family (**Table 2**). The major aglycones were delphinidin (30−39%)

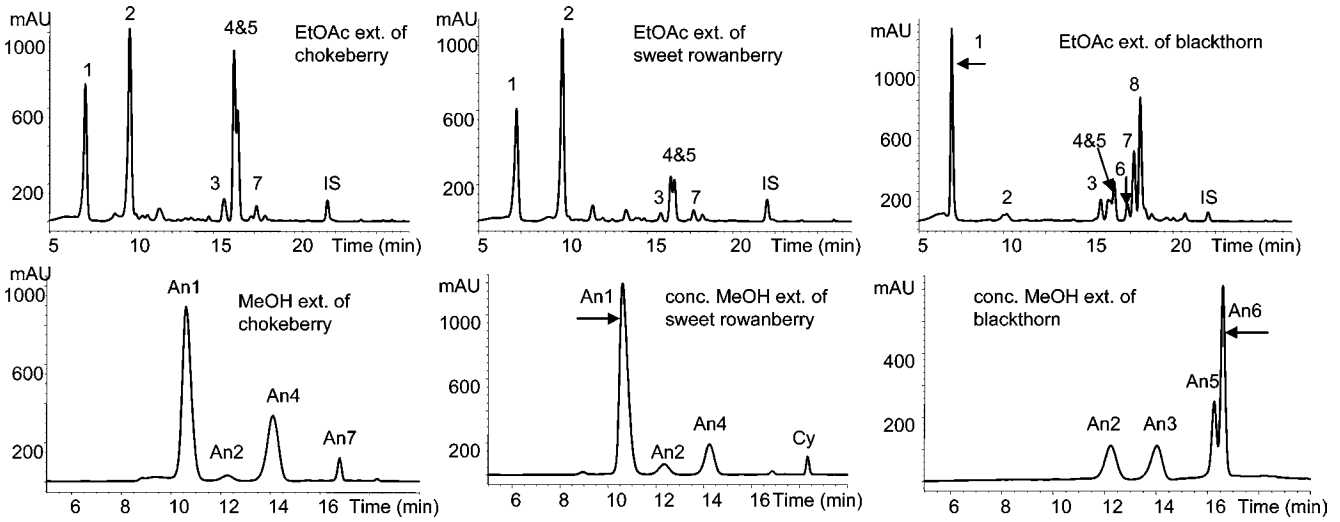

Figure 4. HPLC-DAD chromatograms of berries of the Rosaceae family recorded at 360 nm for the ethyl acetate extracts (EtOAc ext.) and at 520 nm for the methanol extracts (MeOH ext). Peak identification: 1, neochlorogenic acid; 2, chlorogenic acid; 3, quercetin 3-rutinoside; 4, quercetin 3-galactoside; 5, quercetin 3-glucoside; 6, quercetin 3-xyloside; 7, quercetin 3-arabinoside; 8, quercetin 3-rhamnoside; IS, internal standard morin; An1, cyanidin 3-galactoside; An2, cyanidin 3-glucoside; An3, cyanidin 3-rutinoside; An4, cyanidin 3-arabinoside; An5, peonidin 3-glucoside; An6, peonidin 3-rutinoside; An7, cyanidin 3-xyloside; and Cy, cyanidin.

and malvidin (35−45%) in bog whortleberry and delphinidin (34%) and cyanidin (37%) in bilberry but mainly delphinidin (42%) in half-high blueberry (**Table 2**). A similar composition of aglycones was shown for European bilberry (France) and a mixture of wild clones of low-bush blueberries (Canada) (*11*). Cyanidin (44%) and peonidin (56%) occurred at the same levels in cranberry, but cyanidin (99%) dominated clearly in lingonberry (**Table 2**). The environmental, geographical, or other factors in the northernmost latitudes of Finland (Ivalojoki) are correlated with a higher relative content of cyanidin (18 vs 4%) and peonidin in bog whortleberries. In the study of bilberry fruits of various origins, it was shown that cyanidin glycosides were present in slightly higher amounts in berries from the northern latitudes (Norway and Sweden) and delphinidin glycosides from the southern latitudes (Italy, Poland, and Romania) (*43*).

The high contents of (+)-catechin and/or (−)-epicatechin were shown to be a typical feature for the wild berries of *Vaccinium* species. Exceptionally high levels of flavan-3-ols and LMW proanthocyanidins (**Table 2**) were quantified in lingonberry, in agreement with results of a previous study (*6*). (+)-Catechin dominated in the red berries, lingonberry and cranberry, while (−)-epicatechin dominated in blue and black berries, bog whortleberry and bilberry. In European studies on the contents of flavan-3-ols in foodstuffs, (−)-epicatechin dominated in blueberry and cranberry in a Dutch study (*23*) while (+)-catechin dominated in blueberry in a Spanish study (*22*). These inconsistencies might be due to the origin of berry. In the class of insoluble HMW proanthocyanidins, the dominant procyanidin units were characteristics of berries in the Ericaceae family in accordance with available comparison values for blueberry and cranberry (*25*).

Phenolic Compounds in Berries of Rosaceae Family. The selected berries of the Rosaceae family, chokeberry, sweet rowanberry, and blackthorn, lacked ellagitannins typical for berries of *Rubus* and *Fragaria* species of this family (*7*). High contents of chlorogenic and neochlorogenic acid were found in chokeberry and in sweet rowanberry (**Figure 4** and **Table 2**) as found previously in the juice of wild rowanberry (*Sorbus aucuparia* L.) (*44*). In blackthorn, neochlorogenic acid was the dominant conjugated form of hydroxycinnamic acid, as was found in *Prunus* species and other stone fruits (*45*).

In accordance with the previous identification studies, quercetin 3-galactoside and 3-glucoside were found as predominant flavonol glycosides identically in chokeberry and sweet rowanberry (**Figure 4**) (*14*, *44*). Early eluting quercetin glycosides (t_R 13.7 and 14.9 min) were extracted mainly in methanol and were assigned as diglycosides according to the previous identifications (*20*, *44*) (chromatograms not shown). Acid hydrolysis of the ethyl acetate extract of chokeberry, rowanberry, and blackthorn released mainly quercetin as an aglycone (90−95%) and minor amounts of kaempferol, which confirmed the identification of quercetin glycosides (**Figure 4**). Previously, quercetin 3-xyloside, 3-arabinoside, and 3-rhamnoside were isolated and identified from the flowers of blackthorn (*46*), which supports their identification in the berry of blackthorn in the present study (**Figure 4**).

The identical chromatographic profiles of anthocyanins as cyanidin 3-galactoside, 3-glucoside, 3-arabinoside, and 3-xyloside in chokeberry and rowanberry (**Figure 4**) were in accordance with previous identifications (*8*, *14*, *17*). The reddish-black color of chokeberry was parallel with the high content of cyanidin, which was present in 10 times lower amounts in red-colored sweet rowanberry (**Table 2**). In blackthorn, cyanidin and peonidin were typically conjugated with 3-glucoside and 3-rutinoside (*8*).

(−)-Epicatechin was the dominant flavan-3-ol in chokeberry and sweet rowanberry, but (+)-catechin was dominant in blackthorn. Procyanidin units dominated in the insoluble HMW proanthocyanidins (**Table 2**) as in other berries from the Rosaceae family (*25*). High levels of insoluble HMW proanthocyanidins were shown in chokeberry, sweet rowanberry, and blackthorn, consistent with the astringent taste of these berries used as a marker for high tannin (polymeric proanthocyanidins) content (*47*).

Phenolic Compounds in Berries of Empetraceae Family. Two closely related subspecies of crowberry (*Empetrum hermaphroditum* and *Empetrum nigrum*) were analyzed in the family Empetraceae. The growing locations are implied in their naming as northern and southern crowberry. Both crowberries showed the same predominant conjugated hydroxycinnamic acids, flavonol glycosides, and anthocyanins and an almost identical ratio of procyanidin and prodelphinidin of insoluble

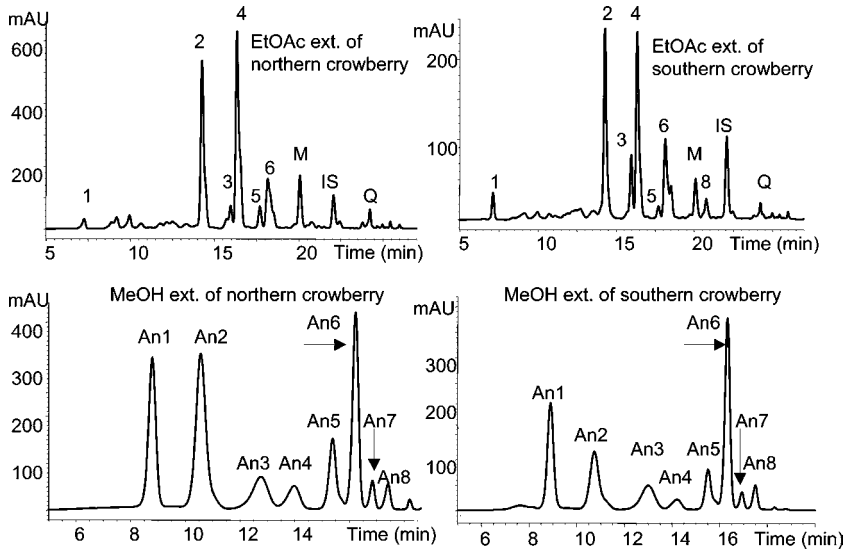

Figure 5. HPLC-DAD chromatograms of berries of the Empetraceae family recorded at 360 nm for the ethyl acetate extracts (EtOAc ext.) and at 520 nm for the methanol extracts (MeOH ext.). Peak identification: 1, neochlorogenic acid; 2, myricetin 3-galactoside (minor 3-glucoside overlapped); 3, quercetin 3-glycoside; 4, quercetin 3-galactoside (minor 3-glucoside overlapped); 5, quercetin 3-arabinoside; 6, quercetin 3-rhamnoside; Q, quercetin; M, myricetin; IS, internal standard; An1, delphinidin 3-galactoside; An2, cyanidin 3-galactoside; An3, petunidin 3-galactoside; An4, cyanidin 3-arabinoside; An5, peonidin 3-galactoside; An6, malvidin 3-galactoside; An7, peonidin 3-arabinoside; and An8, malvidin 3-arabinoside.

HMW proanthocyanidins (**Figure 5** and **Table 2**). Galactose was the most typical sugar conjugate in both flavonol glycosides and anthocyanins in agreement with a previous study on the identification of anthocyanins (*16*). The contents of all phenolic compounds were two or more times higher in northern crowberry as compared to the southern one (**Table 2**), which might be related to the tetraploid chromosomes in the former and diploid chromosomes in the latter. The composition of myricetin (~45%) and quercetin (~55%) was similar in both crowberries in agreement with a previous study (*35*) (**Table 2**). The most distinctive difference was found in the composition of anthocyanidins, where the predominant ones were cyanidin (32%) in northern and malvidin (31%) in southern crowberry.

Phenolic Compounds in Berry of Eleagenaceae Family. Only trace amounts of hydroxycinnamic acids were quantified in sea buckthorn berry. Early eluting quercetin diglycosides were identified according to the study of Häkkinen and Auriola (*20*) and the late eluting isorhamnetin glycosides according to Rösch et al. (*20*, *48*) (**Figure 6**). Deconjugation of flavonol glycosides to aglycones in acid hydrolysis with the similar composition as found in native forms in the ethyl acetate extracts confirmed the identification of quercetin (~30%) and isorhamnetin (~70%) in sea buckthorn. Cell wall-bound myricetin (84 mg/kg) and quercetin (80 mg/kg) were quantified as aglycones after acid hydrolysis of the extraction residue. (+)-Catechin was the major flavan-3-ol in sea buckthorn in agreement with Rösch et al. (*48*). A ratio of procyanidin and prodelphinidin (58:42) showed an almost equal occurrence of both structural units on the proanthocyanidin polymers (**Table 2**). Previously, a respective investigation revealed prodelphinidins (gallocatechins) as the predominating units in sea buckthorn (*48*).

Phenolic Compounds in Berry of Caprifoliaceae Family. Conjugated hydroxycinnamic acids in elderberry were mainly composed of chlorogenic acid. The tentative identification of the three predominant quercetin glycosides was based on their retention times in other berries and quercetin (95%) released in acid hydrolysis of the ethyl acetate extract (**Figure 6**). The occurrence of quercetin 3-rutinoside in elderberry was reported by Macheix et al. (*1*). The predominance of two major

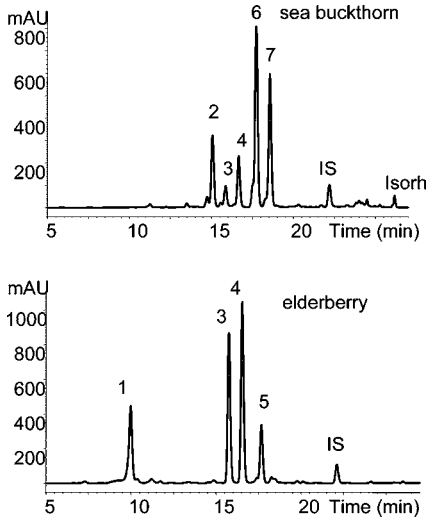

Figure 6. HPLC-DAD chromatograms of ethyl acetate extracts of sea buckthorn (family Elaeagnaceae) and elderberry (family Caprifoliaceae) recorded at 360 nm. Peak identification: 1, chlorogenic acid; 2, quercetin 3-glucosylrhamnoside; 3, quercetin 3-rutinoside; 4, quercetin 3-glucoside; 5, quercetin 3-arabinoside; 6, isorhamnetin 3-glucosylrhamnoside; 7, isorhamnetin 3-rutinoside and 3-glucoside; Isorh, isorhamnetin; and IS, internal standard morin.

anthocyanins in elderberry, cyanidin 3-sambubioside and 3-glucoside, is well-known (*15*, *49*).

In conclusion, we present a modified method in a trial to simultaneously analyze a wide range of phenolic compounds in their different conjugated forms in berries. Various chromatograms provide distinctive profiles for the studied berries. The extraction procedure, optimized to provide a satisfactory qualitative and quantitative result of the free and conjugated hydroxycinnamic acid forms, flavonols and anthocyanidins, led to an underestimation of the contents of flavan-3-ols and LMW proanthocyanidins.

Generally, the most common phenolic compounds in the studied berries were quercetin 3-glucoside and cyanidin 3-glucoside. A comparison of berry species of the same family

revealed similarities with respect to the distribution of conjugated phenolic compounds but mainly variations in the chromatographic profiles of the conjugates and the relative levels of aglycones, especially in the case of anthocyanins. These data are useful for the identification of different berries in studies on the authenticity of berry raw material in the food industry. Detailed studies are needed on the possible effect of maturity, environmental factors, or geographical origin on the absolute and relative levels of various phenolic aglycones and their conjugated forms in berries.

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Supporting Information Available: Table on the distribution of the predominant conjugated hydroxycinnamic acids, flavonol glycosides, and anthocyanins in 18 berries of six families. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Macheix, J.-J.; Fleuriet, A.; Billot, J. *Fruit Phenolics*; CRC: Boca Raton, Florida, 1990; pp 1−126.
(2) Haslam, E. Natural polyphenols (vegetable tannins) as drugs: Possible modes of action. *J. Nat. Prod.* **1996**, *59*, 205−215.
(3) Lairon, D.; Amiot, M. J. Flavonoids in food and natural antioxidants in wine. *Curr. Opin. Lipidol.* **1999**, *10*, 23−28.
(4) Morton, L. W.; Abu-Amsha Caccetta, R.; Puddey, I. B.; Croft, K. D. Chemistry and biological effects of dietary phenolic compounds: relevance to cardiovascular disease. *Clin. Exp. Pharmacol. Physiol.* **2000**, *27*, 152−159.
(5) Parr, A. J.; Bolwell, G. P. 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.* **2000**, *80*, 985−1012.
(6) Kähkönen, M. P.; Hopia, A. I.; Heinonen, M. Berry phenolics and their antioxidant activity. *J. Agric. Food Chem.* **2001**, *49*, 4076−4082.
(7) Häkkinen, S. H.; Kärenlampi, S. O.; Mykkänen, H. M.; Heinonen, I. M.; Törrönen, A. R. Ellagic acid content in berries: Influence of domestic processing and storage. *Eur. Food Res. Technol.* **2000**, *212*, 75−80.
(8) Espin, J. C.; Soler-Rivas, C.; Wichers, H. J.; García-Viguera, C. Anthocyanin-based natural colorants: A new source of antiradical activity for foodstuff. *J. Agric. Food Chem.* **2000**, *48*, 1588−1592.
(9) Määttä, K. R.; Kamal-Eldin, A.; Törrönen, A. R. High-performance liquid chromatography (HPLC) analysis of phenolic compounds in berries with diode array and electrospray ionization mass spectrometric (MS) detection: *Ribes* species. *J. Agric. Food Chem.* **2003**, *51*, 6736−6744.
(10) Jaakola, L.; Määttä, K.; Pirttilä, A. M.; Törrönen, R.; Kärenlampi, S.; Hohtola, A. Expression of genes involved in anthocyanin biosynthesis in relation to anthocyanin, proanthocyanidin, and flavonol levels during bilberry fruit development. *Plant Physiol.* **2002**, *130*, 729−739.
(11) Kalt, W.; McDonald, J. E.; Richer, R. D.; Lu, X. Anthocyanin content and profile within and among blueberry species. *Can. J. Plant Sci.* **1999**, *79*, 617−623.
(12) Andersen, Ø. M. Chromatographic separation of anthocyanins in cowberry (lingonberry) *Vaccinium vitis-idaea* L. *J. Food Sci.* **1985**, *50*, 1230−1232.
(13) Huopalahti, R.; Järvenpää, E. P.; Katina, K. A novel solid-phase extraction-HPLC method for analysis of anthocyanins and organic acid compositions of Finnish cranberry. *J. Liq. Chromatogr. Relat. Technol.* **2000**, *23*, 2695−2701.
(14) Zheng, W.; Wang, S. Y. Oxygen radical absorbing capacity of phenolics in blueberries, cranberries, chokeberries, and lingonberries. *J. Agric. Food Chem.* **2003**, *51*, 502−509.
(15) Chandra, A.; Rana, J.; Li, Y. Q. Separation, identification, quantification, and method validation of anthocyanins in botanical supplement raw materials by HPLC and HPLC-MS. *J. Agric. Food Chem.* **2001**, *49*, 3515−3521.
(16) Kärppä, J.; Kallio, H.; Peltonen, I.; Linko, R. Anthocyanins of crowberry, *Empetrum nigrum* coll. *J. Food Sci.* **1984**, *49*, 634−636.
(17) Pyysalo, H.; Kuusi, T. Phenolic compounds form the berries of mountain ash, *Sorbus aucuparia*. *J. Food Sci.* **1974**, *39*, 636−638.
(18) Azar, M.; Verette, E.; Brun, S. Identification of some phenolic compounds in bilberry juice *Vaccinium myrtillus*. *J. Food Sci.* **1987**, *52*, 1255−1257.
(19) Herrmann, K. Occurrence and content of hydroxycinnamic and hydroxybenzoic acid compounds in foods. *Crit. Rev. Food Sci. Nutr.* **1989**, *28*, 315−347.
(20) 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.
(21) Yan, X.; Murphy, B. T.; Hammond, G. B.; Vinson, J. A.; Neto, C. C. Antioxidant activities and antitumor screening of extracts from cranberry fruit (*Vaccinium macrocarpon*). *J. Agric. Food Chem.* **2002**, *50*, 5844−5849.
(22) de Pascual-Teresa, S.; Santos-Buelga, C.; Rivas-Gonzalo, J. C. Quantitative analysis of flavan-3-ols in Spanish foodstuffs and beverages. *J. Agric. Food Chem.* **2000**, *48*, 5331−5337.
(23) Arts, I. C.; van De Putte, B.; Hollman, P. C. Catechin contents of foods commonly consumed in The Netherlands. 2. Tea, wine, fruit juices, and chocolate milk. *J. Agric. Food Chem.* **2000**, *48*, 1752−1757.
(24) Gu, L.; Kelm, M. A.; Hammerstone, J. F.; Beecher, G.; Holden, J.; Haytowitz, D.; Gebhardt, S.; Prior, R. L. Concentrations of proanthocyanidins in common foods and estimations of normal consumption. *J. Nutr.* **2004**, *134*, 613−617.
(25) Foo, L. Y.; Porter, L. J. The structure of tannins of some edible fruits. *J. Sci. Food Agric.* **1981**, *32*, 711−716.
(26) Gu, L.; Kelm, M.; Hammerstone, J. F.; Beecher, G.; Cunningham, D.; Vannozzi, S.; Prior, R. L. Fractionation of polymeric procyanidins from lowbush blueberry and quantification of procyanidins in selected foods with an optimized normal-phase HPLC-MS fluorescent detection method. *J. Agric. Food Chem.* **2002**, *50*, 4852−4860.
(27) Tura, D.; Robards, K. Sample handling strategies for the determination of biophenols in food and plants. *J. Chromatogr. A* **2002**, *975*, 71−93.
(28) Määttä, K.; Kamal-Eldin, A.; Törrönen, R. Phenolic compounds in berries of black, red, green, and white currants (*Ribes* sp.). *Antioxid. Redox Signaling* **2001**, *3*, 981−993.
(29) Mattila, P.; Kumpulainen, J. Determination of free and total phenolic acids in plant-derived foods by HPLC with diode-array detection. *J. Agric. Food Chem.* **2002**, *50*, 3660−3667.
(30) Treutter, D.; Santos-Buelga, C.; Gutmann, M.; Kolodziej, H. Identification of flavan-3-ols and procyanidins by high-performance liquid-chromatography and chemical-reaction detection. *J. Chromatogr. A* **1994**, *667*, 290−297.
(31) de Pascual-Teresa, S.; Treutter, D.; Rivas-Gonzalo, J. C.; Santos-Buelga, C. Analysis of flavanols in beverages by high-performance liquid chromatography with chemical reaction detection. *J. Agric. Food Chem.* **1998**, *46*, 4209−4213.
(32) Bartolomé, B.; Hernández, I.; Bengoechea, M. L.; Quesada, C.; Gómez-Cordovés, C.; Estrella, I. Determination of some structural features of procyanidins and related compounds by photodiode-array detection. *J. Chromatogr. A* **1996**, *723*, 19−26.

(33) Porter, L. J.; Hrstich, L. N.; Chan, B. G. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry* **1986**, *25*, 223−230.
(34) Rohr, G. E.; Meier, B.; Sticher, O. Analysis of procyanidins. In *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier Science B. V.: New York, 2000; pp 497−569.
(35) 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.* **1999**, *47*, 2274−2279.
(36) 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.* **1999**, *32*, 345−353.
(37) Slimestad, R.; Solheim, H. Anthocyanins from black currants (*Ribes nigrum* L.). *J. Agric. Food Chem.* **2002**, *50*, 3228−3231.
(38) Harborne, J. B.; Hall, E. Plant polyphenols-XIII. The systematic distribution and origin of anthocyanins containing branched trisaccharides. *Phytochemistry* **1964**, *3*, 453−463.
(39) Iversen, C. K. Black currant nectar: Effect of processing and storage on anthocyanin and ascorbic acid content. *J. Food Sci.* **1999**, *64*, 37−41.
(40) Øydvin, J. Inheritance of four cyanidin-3-glycosides in red currant. *Hortic. Res.* **1974**, *14*, 1−7.
(41) Markham, K. R. *Techniques of Flavonoid Identification*; Academic Press: New York, 1982; pp 36−39.
(42) Häkkinen, S. H.; Törrönen, A. R. Content of flavonols and selected phenolic acids in strawberries and *Vaccinium* species: Influence of cultivar, cultivation site and technique. *Food Res. Int.* **2000**, *33*, 517−524.
(43) Martinelli, E. M.; Baj, A.; Bombardelli, E. Computer-aided evaluation of liquid-chromatographic profiles for anthocyanins in *Vaccinium myrtillus* fruits. *Anal. Chim. Acta* **1986**, *191*, 275−281.
(44) Gil-Izquierdo, A.; Mellenthin, A. Identification and quantitation of flavonols in rowanberry (*Sorbus aucuparia* L.) juice. *Eur. Food. Res. Technol.* **2001**, *213*, 12−17.
(45) Möller, B.; Herrmann, K. Quinic acid esters of hydroxycinnamic acids in stone and pome fruits. *Phytochemistry* **1983**, *22*, 477−481.
(46) Olszewska, M.; Wolbis, M. Further flavonoids from the flowers of *Prunus spinosa* L. *Acta Pol. Pharm.* **2002**, *59*, 133−137.
(47) Noble, A. C. Astringency and bitterness of flavonoid phenols. In *Chemistry of Taste: Mechanisms, Behaviors, and Mimics*; Given, P., Paredes, D., Eds.; American Chemical Society: Washington, DC, 2002; pp 192−201.
(48) Rösch, D.; Bergmann, M.; Knorr, D.; Kroh, L. W. Structure-antioxidant efficiency relationships of phenolic compounds and their contribution to the antioxidant activity of sea buckthorn juice. *J. Agric. Food Chem.* **2003**, *51*, 4233−4239.
(49) Goiffon, J.-P.; Mouly, P. P.; Gaydou, E. M. Anthocyanic pigment determination in red fruit juices, concentrated juices syrups using liquid chromatography. *Anal. Chim. Acta* **1999**, *382*, 39−50.

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