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Catechins and Procyanidins in Berries of *Vaccinium* Species and Their Antioxidant Activity

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The fractions of monomeric catechins and the fractions of dimeric and trimeric procyanidins were extracted and concentrated from wild berries of *Vaccinium* species to study their antioxidant activities. The compositions of the fractions were analyzed using high-performance liquid chromatography combined with diode-array and electrospray ionization mass spectrometric detection. Rare A-type dimers and trimers were identified as the predominant procyanidins in wild lingonberry, cranberry, bilberry, and bog whortleberry. Lingonberry and cranberry catechin and procyanidin fractions as well as bog whortleberry catechin fraction were good scavengers of radicals in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test and more efficient than the respective bilberry fractions. Bog whortleberry procyanidin fraction was less active, this being mainly due to the lower content of these compounds. Fractions from lingonberry, cranberry, and bilberry were equally efficient in inhibiting the oxidation of methyl linoleate emulsion, but differences among the berries were found in their abilities to inhibit low-density lipoprotein (LDL) oxidation. Catechins, the monomers, exhibited comparable activity to the fractions containing dimers and trimers in inhibiting the oxidation of methyl linoleate emulsion and human LDL. Bog whortleberry catechins were excellent antioxidants toward the oxidation of human LDL. Radical scavenging and antioxidant activities of *Vaccinium* berry fractions were attributable to the their composition of catechins and procyanidins. In conclusion, *Vaccinium* catechins as well as dimeric and trimeric procyanidins provide substantial antioxidant protection.

KEYWORDS: Food analysis; berries; phenolic compounds; HPLC; proanthocyanidins; antioxidants; radical scavengers; LDL

INTRODUCTION

Vaccinium species are low-growing, perennial herbs found in the temperate climate zones. In Scandinavia, wild berries of lingonberry (*V. vitis-idaea* L.), cranberry (*V. oxycoccos* L.), bilberry (*V. myrtillus* L.), and occasionally bog whortleberry (*V. uliginosum* L.) are consumed fresh or in juices, jams, and jellies. We have recently studied the composition and contents of flavonoids in berries of *Vaccinium* species, since these compounds are believed to possess health-promoting effects (*1*−*4*). Consumption of flavonoid-rich plant foods has been claimed to protect against cardiovascular diseases and certain cancers, such as lung cancer (*5*). It is known that the oxidation of low-density lipoproteins (LDL) is associated with cardiovascular diseases (*6*, *7*), and thus flavonoids, compounds possessing antioxidant activity, are postulated to have potential benefits in the prevention of these diseases.

The group of flavonoids includes structurally related compound classes named flavonol glycosides, anthocyanins, flavan-3-ols, and proanthocyanidins. Catechins of the flavan-3-ol class have dihydroxy substituents on their B ring and procyanidins of the proanthocyanidin class are the respective polymerized molecules (**Figure 1**). The antioxidant activities of flavonoids as well as of plant extracts have been the subject of several studies in the past years (*8*). Berries of the *Vaccinium* species have been shown to possess radical scavenging capacity in various in vitro models using assays of the oxygen radical absorbance capacities (ORAC), the ferric reducing antioxidant power (FRAP), the total oxidant scavenging capacity (TOSC), and the free radical scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical as well as antioxidant capacities in inhibiting oxidation of methyl linoleate, liposomes, and human low-density lipoprotein (LDL) (*2*, *9*−*15*). Among flavonoids, anthocyanins were the greatest contributors to the

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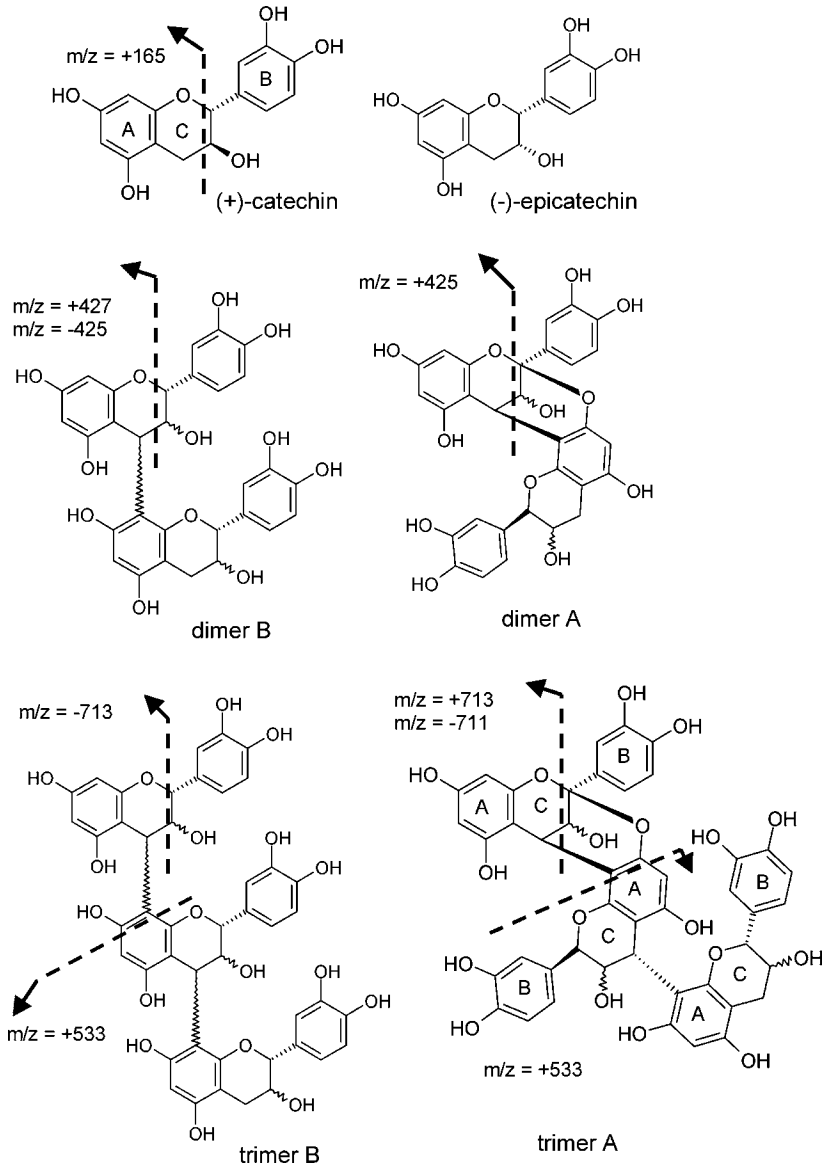

Figure 1. Structures of catechins and low molecular weight procyanidins. Dashed arrows refer to the favored formation of positive and negative fragment ions in MS–MS.

antioxidant capacity in *Vaccinium* species, except in lingonberries, in which catechins and procyanidins dominated (*2*, *12*, *14*). In one study cranberry (*10*) and in another study lingonberry (*15*) exhibited the highest antioxidant capacity among the common fruits in the TOSC assay. However, the contribution of catechins and procyanidins to the antioxidant activity of *Vaccinium* species has not been as extensively studied as that of other flavonoids.

The composition and content of procyanidins have been studied in common plant foods (*16*, *17*). The universal B-type procyanidins have a single link between structural units of catechins, whereas the rare A-type procyanidins are double linked (**Figure 1**). Cultivated cranberry (*V. macrocarpon* Ait.) and wild lingonberry contain both A- and B-type procyanidins (*16*, *18*, *19*), whereas primary B-type procyanidins were identified in wild (*V. angustifolium* Ait.) and cultivated blueberries (*V. corymbosum* L., *V. ashei* L.) (*16*, *20*, *21*). The procyanidin composition of other berries of *Vaccinium* species is not known.

In this study, fractions of catechins and procyanidins were extracted and concentrated from berries of the wild Scandinavian species of lingonberry, cranberry, bilberry, and bog whortleberry for the tentative identification and the determination of their antioxidant activity. Radical-scavenging and antioxidant capacities were studied using the DPPH test and models based on oxidation of methyl linoleate and LDL. These methods have been previously applied to other berry fractions, e.g. *Vaccinium* anthocyanins, in our laboratory (*22*). The identification tools were on-line UV−visible spectra of diode-array detection (DAD) for the subgroup specification and electrospray ionization mass spectrometry (MS) for the elucidation of the molecular weight and subsequent tandem MS for the further restricted structural information.

MATERIALS AND METHODS

Samples, Standards, and Lipid Phases. Wild lingonberry (*V. vitis-idaea* L.), cranberry (*V. oxycoccos* L.), bilberry (*V. myrtillus* L.), and bog whortleberry (*V. uliginosum* L.) were purchased from a Finnish supplier of berries and berry products (Kiantama Ltd). The berries were frozen at −24 ± 2 °C prior to extraction. (+)-Catechin, (−)-epicatechin, and human LDL were purchased from Sigma Chemical Co. (St Louis, MO). Methyl linoleate (MeLo) was obtained from Nu Check Prep (Elysina, MN), and Emultop (partially hydrolyzed soybean lecithin) was a gift from Lucas Meyer GmbH (Hamburg, Germany).

Extraction and Fractionation of Catechins and Procyanidins. Frozen berries (600 g) were crushed to a fine powder, 150 mL of ascorbic acid (1 g/100 mL) in water was added, and phenolic acids, catechins, dimeric and trimeric procyanidins, and flavonol glycosides were subsequently extracted with ethyl acetate (600 mL) by shaking for 2 h at room temperature. The ethyl acetate extracts were liquid/liquid extracted twice with 200 mL of sodium acetate buffer (0.1 M, pH 7.0) and then with 200 mL of water. The extraction and purification protocol was modified from that used in our previous studies on semiquantification of catechins and low molecular weight proanthocyanidins from berries (*3*, *4*). After evaporation to dryness under vacuum at 35 °C, the purified ethyl acetate extract was reconstituted into 10 mL with methanol for fractionation in an open Sephadex LH-20 (Amersham Pharmacia) column (30 × 2 cm) equilibrated with sodium acetate buffer (0.1 M, pH 7.0). The following fractionation protocol was modified from that described in Puupponen-Pimiä et al. (*23*). The extract was mixed with an equal volume of sodium acetate buffer, the precipitating components were eliminated by centrifugation, and the supernatant was applied to the column. Residues of phenolic acids were removed by elution with 40 mL of sodium acetate buffer and 50 mL of 30% methanol in water. Catechins were eluted with 50 mL of 60% methanol in water, and dimeric and trimeric proanthocyanidins were retrieved with 50 mL of pure methanol. Flavonol glycosides co-occurred in these fractions. The extraction and fractionation procedures were repeated one to three times to collect sufficient amounts of catechins and procyanidins. The fractions were concentrated by vacuum evaporation and further purified with the preparative HPLC method described by Kähkönen et al. (*22*). No phenolic compounds other than catechins and procyanidins were detected in the purified fractions.

Characterization and Quantification of Catechins and Procyanidins. Catechins and procyanidins in the fractions were characterized by HPLC combined with electrospray ionization mass spectrometric detection (LC−MS). Adequate separation was achieved 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) by running a 20-min linear gradient from 5 to 30% acetonitrile in 1% formic acid in water at a flow rate of 0.5 mL/min. The parameters for positive ionization were adapted from Häkkinen et al. (*24*) and for negative ionization from Mämmelä et al. (*25*). The system used for LC−MS analysis was a Finnigan MAT LCQ ion trap mass spectrometer (San Jose, CA) equipped with a Rheos 400 HPLC pump (Danderyd, Sweden). MS revealed the positive or negative molecular ions, and MS−MS and the subsequent MS−MS−MS were used to break down the most abundant species with collision-induced dissociation. Ionization conditions were optimal for the standards of (+)-catechin and (−)-epicatechin.

HPLC combined with diode-array detection (LC−DAD) was used for spectral analysis and quantification. For the better separation of dimeric and trimeric procyanidins, a new gradient of acetonitrile in 5% formic acid was applied. The concentration of acetonitrile was 5% for 5 min and then a linear gradient to 20% was run (5−35 min) at a flow rate of 0.5 mL/min. Since separate elution programs were used in LC−DAD and LC−MS analyses, the order of retention was valid but the exact times were not. Peaks in the chromatograms were primarily identified by the typical and identical UV-spectra of catechins and procyanidins (*26*). Procyanidins were further assigned according to the LC−MS results. (+)-Catechin and (−)-epicatechin were used as standards in identification according to the retention times and quantification using the response factors. The response factors were from twice-prepared fresh solutions in the concentration range 2−250 mg/L. Procyanidins were tentatively quantified using the response factor of (−)-epicatechin.

Antioxidant Activity Testing. Radical scavenging activity using the DPPH test and antioxidant activity toward the oxidation of both MeLo emulsion and human LDL were investigated as described by Kähkönen et al. (*22*). The ability of the catechin and procyanidin fractions to act as free radical scavengers against DPPH radical was tested spectrophotometrically by measuring the decrease of the absorbance at 517 nm after adding the antioxidant solution. A volume of 2950 μL of 0.1 mM methanolic DPPH solution was mixed in a cuvette with 50 μL of the fraction in methanol (final concentrations 4.2 and 8.3 μg of the dried fraction/mL). The absorption was monitored at 15 s intervals for 5 min. The results are expressed as the percentage of radicals scavenged after 4 min of reaction time.

The experiments in 10% oil-in-water emulsion were carried out using 0.400 g of MeLo and 3.6 mL of 1% (w/v) Emultop in Milli-Q water. Catechin and procyanidin fractions in methanol were added at levels of 100 and 500 ppm and the solvent was removed by nitrogen flushing. Then MeLo and the emulsifier solution were added for the preparation of emulsion. Oxidation of MeLo emulsions was carried out in the dark at 40 °C. To study LDL oxidation, human LDL was diluted to a protein concentration of 0.2 mg/mL using 0.01 M sodium phosphate buffer, pH 7.4, containing 0.15 M NaCl. The catechin and procyanidin fractions were added in ethanolic solution (final concentrations 2.5 and 7.5 μg of the dried fraction/mL), and ethanol was removed by nitrogen flushing. Then, LDL solution, phosphate buffer, and copper sulfate were added to form the reaction mixture. The antioxidant activity was expressed as percent inhibition of either the formation of conjugated diene hydroperoxides measured spectrophotometrically (Perkin-Elmer lambda 15 UV−vis spectrophotometer, Norwalk, CT) at 234 nm after 72 h of oxidation (MeLo emulsion) or the inhibition of the formation of hexanal using static headspace gas chromatography (Perkin-Elmer Autosystem XL gas chromatograph equipped with Perkin-Elmer HS 40XL headspace sampler, Shelton, CT) after 2 h incubation (LDL oxidation).

RESULTS

Identification of Catechins and Procyanidins. Catechins and procyanidins were extracted and concentrated into two fractions from wild lingonberry, cranberry, bilberry, and bog whortleberry. The fractionation was based on mobility in Sephadex LH-20 gel in elution with 60% aqueous methanol (catechin fraction) and subsequent elution with 100% methanol (procyanidin fraction). The LC−MS data of the fractions were screened for protonated positive $(M + H)^+$ and deprotonated $(M - H)^-$ negative molecular ions of catechins, dimers B, trimers B, dimers A and trimers A (**Table 1**, **Figures 2** and **3** for lingonberry fractions). The typical fragmentation patterns of molecular ions in MS−MS and further in MS−MS−MS were screened and are shown in **Table 1**. Previous LC−MS studies assisted in the interpretation of the fragmentation patterns of catechins and procyanidins (*27*−*29*). The peaks in LC−DAD chromatograms at 280 nm exhibited the typical and identical UV−visible spectra of catechins and low molecular weight procyanidins (**Figure 2** and **3**) (*26*). (+)-Catechin and (−)-epicatechin were identified according to the retention times and the on-line UV−visible spectra of standards in LC−DAD. The LC−MS data confirmed the presence of catechins and one additional dimer B in fractions eluted with 60% methanol (**Figure 2**, **Table 1**). **Figure 3** shows the diverse composition of dimeric and trimeric lingonberry procyanidins in LC−MS and LC−DAD chromatograms. The typical MS−MS fragment ions of B-type dimers were formed at *m*/*z* 427, 409 (positive ions), and 425 (negative ion) by the loss of neutral mass units 152 and 170 (152 + water) as shown in **Figure 1**. The same mass units were also eliminated from the upper units of the structures of dimers A as well as trimers B and A in fragmentation both in the positive and negative ionization modes (**Table 1**), which means that this fragmentation pattern may be a characteristic attribute for these structures (**Figure 1**). There was another favored fragmentation pattern; both A- and B-type trimers exhibited a positive MS−MS fragment ion at *m*/*z* 533 formed by the loss of one catechin unit, as shown in **Figure 1**.

Composition of the Fractions. Table 2 shows the composition of the catechin and procyanidin fractions extracted and concentrated from *Vaccinium* berries. Tentative quantification

Table 1. Identification of Catechins and Procyanidins in Berries of *Vaccinium* Species Using LC–MS, MS–MS, and MS–MS–MS Data in Positive and Negative Modes

	positive ions			negative ions			
Rt_R[a]	$(M + H)^+$	MS–MS[b]	MS–MS–MS	$(M - H)^-$	MS–MS[b]	MS–MS–MS	tentative identification
0.68	579	**427**, 409, 301	275	577	425	407	dimer B
0.81	865	**713**, 533	695, 425	863	**711**, 695, 575	693, 559	trimer A
0.81	291	165, **139**	ND	289	245	203	(+)-catechin
0.89	867	**533**, 283	408	865	713, **695**, 575	543	trimer B
0.91	579	**427**, 409	287	577	425	407	dimer B
1.00	865	713, **533**	515, 407	ND	711, **575**	539, 449	trimer A
1.00	291	165, **139**	ND	289	245	203	(-)-epicatechin
1.04	865	713, **533**	407, 287	863	711	693, 559	trimer A
1.06	867	849, 579, **577**	ND	865	695	543	trimer B
1.09	579	409	287	577	425	407	dimer B
1.15	865	545	420	863	711	693	trimer A
1.24	577	437, **425**	287	575	449	287	dimer A
1.34	579	409	287	ND	ND	ND	dimer B
1.40	577	425	287	575	449	287	dimer A

[a] Relative retention time compared to (–)-epicatechin in LC–DAD run. [b] The molecular ions $(M + H)^+$ or $(M - H)^-$ were fragmented in MS–MS by the loss of a neutral mass unit to charged ions (charge depends on the ionization mode), of which the major ion (in bold) was further fragmented in MS–MS–MS. Only the most intense ions (relative abundance > 50%) are shown.

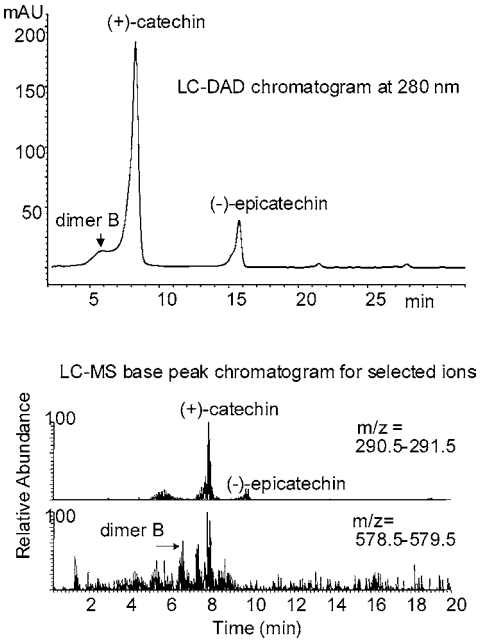

Figure 2. LC–DAD and LC–MS chromatograms of lingonberry catechin fraction.

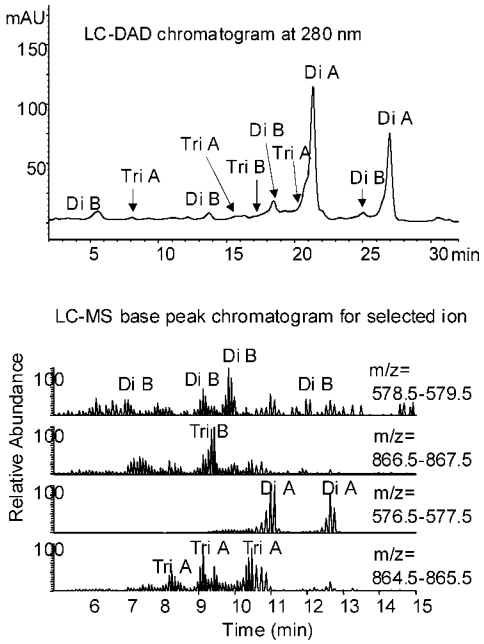

Figure 3. LC–DAD and LC–MS chromatograms of lingonberry procyanidin fraction. Abbreviations: Di, dimer; Tri, trimer.

was made using the standards (+)-catechin and (−)-epicatechin, which showed almost identical molar absorbances in LC−DAD. The best available standards for procyanidins would be the original isolates of A-type and B-type procyanidins, since there is variation in the molar absorbances due to the chain length and the type of bond (*30*). However, relevant quantification of procyanidins for comparison between the berry fractions was achieved using (−)-epicatechin as the representative standard. The total contents of catechins with a co-occurring dimer B in the catechin fractions varied from 728 μg/mg in lingonberry to 864 μg/mg in cranberry. Within the catechins, (+)-catechin dominated in lingonberry and cranberry (as sum of both fractions) and (−)-epicatechin in bilberry and bog whortleberry (**Table 2**), in agreement with our previous results (*3*). The composition of procyanidins in the fractions reflected the original composition of the berries and to some extent the extractability of dimers and trimers to ethyl acetate (*30*). Procyanidins were mainly composed of A-type dimers in lingonberry and A-type trimers in bog whortleberry and bilberry. The contents of procyanidins in the procyanidin fractions varied from 194 μg/mg in cranberry to 818 μg/mg in lingonberry. In cranberries, however, a substantial amount of catechins (496 μg/mg) was present in the procyanidin fraction. Cranberry catechins were not fully eluted with 60% methanol and were subsequently eluted in the procyanidin fraction.

Antioxidant Activity of Catechin and Procyanidin Fractions. Radical scavenging activity against DPPH of the catechin and procyanidin fractions was dose dependent in the concentration ranges used (**Table 3**). Lingonberry and cranberry catechin and procyanidin fractions were good scavengers of radicals (>50% of radicals scavenged in 4 min) only at the higher concentration of 8.3 μg/mL and were more efficient than the respective bilberry fractions. The bog whortleberry catechins contributed substantially to the radical scavenging activity, while the activity attributable to the fraction composed of procyanidin dimers and trimers was rather minor, due to the lower total amounts of these compounds present (**Table 2**).

Table 2. Contents[a] of Catechins and Procyanidins in Fractions of *Vaccinium* Berries

		lingonberry		cranberry		bog whortleberry		bilberry	
	Rt_R[b]	Ca Fr[c]	ProC Fr	Ca Fr	Ca &ProC Fr	Ca Fr	ProC Fr	Ca Fr	ProC Fr
(+)-catechin	0.81	584	ND[d]	417	496	70	ND	215	ND
(-)-epicatechin	1.00	88	ND	447	ND	639	ND	400	ND
dimer B	0.68	56	27	ND	13	ND	ND	ND	ND
dimer B	0.91	ND	23	ND	21	36	20	123	47
dimer B	1.09	ND	64	ND	ND	ND	47	ND	55
dimer B	1.34	ND	12	ND	ND	ND	ND	ND	50
dimer A	1.24	ND	303	ND	52	ND	33	ND	141
dimer A	1.40	ND	173	ND	12	ND	5	ND	28
trimer B	0.89	ND	21	ND	ND	ND	ND	ND	ND
trimer B	1.06	ND	42	ND	31	ND	ND	ND	35
trimer A	0.81	ND	16	ND	ND	ND	5	ND	14
trimer A	1.00	ND	50	ND	31	ND	30	ND	68
trimer A	1.04	ND	ND	ND	34	ND	76	ND	274
trimer A	1.15	ND	87	ND	ND	ND	39	ND	ND
total		**728**	**818**	**864**	**690**	**745**	**255**	**738**	**712**

[a] μg/mg of dried fraction. [b] Relative retention time compared to (–)-epicatechin in LC–DAD run. [c] Abbreviations: Ca Fr, fraction composed of catechins; ProC Fr, fraction composed of procyanidins. [d] ND, not detected.

Table 3. Free Radical Scavenging Ability (DPPH Test) and Antioxidant Activity toward Oxidation of Methyl Linoleate Emulsion and Human LDL of Catechin and Procyanidin Fractions Extracted from Berries of *Vaccinium* Species

	DPPH[a]		emulsion[b]		LDL[c]	
	4.2 μg/mL	8.3 μg/mL	100 ppm	500 ppm	2.5 μg/mL	7.5 μg/mL
			Lingonberry			
Ca Fr[d]	40 ± 0	65 ± 1	85 ± 0	87 ± 2	13 ± 7	94 ± 0
ProC Fr	42 ± 0	74 ± 0	84 ± 0	86 ± 0	24 ± 2	96 ± 1
			Cranberry			
Ca Fr	39 ± 0	71 ± 1	80 ± 0	NA[e]	–17 ± 4	88 ± 0
Ca & ProC Fr	31 ± 0	58 ± 2	87 ± 1	84 ± 1	–27 ± 4	86 ± 2
			Bilberry			
Ca Fr	23 ± 1	47 ± 2	83 ± 1	NA	–20 ± 1	94 ± 0
ProC Fr	24 ± 1	47 ± 2	85 ± 1	84 ± 3	–16 ± 1	92 ± 1
			Bog Whortleberry			
Ca Fr	35 ± 0	58 ± 2	71 ± 1	66 ± 6	92 ± 4	96 ± 1
ProC Fr	10 ± 1	20 ± 1	39 ± 3	73 ± 5	–7 ± 5	–8 ± 5

[a] Percentage of scavenged radicals (%) after 4 min of reaction time; mean ± SD, $n = 3$. [b] Inhibition (%) of formation of conjugated diene hydroperoxides after 72 h of incubation; $n = 2$. [c] Inhibition (%) of formation of hexanal after 2 h of incubation; $n = 2$. [d] Abbreviations: Ca Fr, fraction composed of catechins; ProC Fr, fraction composed of procyanidins. [e] NA, not analyzed.

While radical scavenging activity reflects the structure–activity relationship, i.e., the possibility to act as an antioxidant, other methods targeted for inhibition of oxidation are needed to evaluate the food preservative potential or health effects of antioxidants. All catechin and procyanidin fractions extracted and concentrated from the berries were efficient inhibitors of the oxidation of methyl linoleate emulsion (**Table 3**). The antioxidant effect was not dose dependent at the concentration levels used, as, in general, more than 80% of the formation of lipid hydroperoxides was inhibited with a concentration of 100 ppm. Only bog whortleberry procyanidin fraction exhibited dose dependence, and this was due to the low content of procyanidins present. Catechins, the monomers, were as active in inhibiting the oxidation of methyl linoleate emulsion as the fractions containing dimers and trimers.

Bog whortleberry catechins were excellent antioxidants (inhibition > 90%) against the oxidation of human LDL at both concentrations (2.5 and 7.5 μg/mL), whereas the bog whortleberry procyanidins promoted oxidation (**Table 3**). In the case of the other berries, the catechin fractions showed comparable activity in inhibiting oxidation of LDL as the fractions containing dimers and trimers. Lingonberry and bilberry followed by cranberry catechins and procyanidins inhibited LDL oxidation efficiently at the higher concentration. However, bilberry and cranberry fractions promoted oxidation at the lower concentration.

DISCUSSION

Rare A-type low molecular weight procyanidins were detected in wild lingonberry, cranberry, bilberry, and bog whortleberry and were present at higher levels than the more common B-type procyanidins. Previously, the three-dimensional structures of A-type dimers and trimers have been identified in cultivated cranberry and lingonberry of *Vaccinium* species (*18*, *19*). In the more common plant foods, A-type linkages have been detected in plums, avocados, and peanuts (*17*). This unique double linked chain structure of flavonoids aroused special interest, because it was suspected to contribute to antiadhesion activity against bacteria (*31*) and to the antiviral effects of these food products (*32*). In the present study, Scandinavian bilberry and bog whortleberry were shown to contain A-type procyanidins for the first time. Previously, only primary B-type forms have been reported in other bluish-black colored *Vaccinium* species (*20*, *21*). In our previous study, the content of catechins was 10 times higher in lingonberry (250 mg/kg fresh weight) than in the other *Vaccinium* species (cranberry, 30 mg/kg; bilberry, 75 mg/kg; and bog whortleberry, 20–30 mg/kg) (*3*). The contents of procyanidins were even 30 times higher in lingonberry compared to those present in other berries (*2*, *3*). In the present study, these catechins and procyanidins were extracted and concentrated from the wild *Vaccinium* berries to study their antioxidant activity.

Flavonoids with adjacent dihydroxy substituents on the B ring have been shown to be effective in radical scavenging (*11*). In the group of flavonoids, this catechol unit is found in quercetin of flavonols, cyanidin of anthocyanidins, catechins of flavan-3-ols, and procyanidins of proanthocyanidins. In the present study, both catechin as well as dimeric and trimeric procyanidin fractions possessed radical scavenging activity at the higher concentration level tested. According to Ursini et al. (*33*), dimeric and oligomeric B-type procyanidins possessed greater scavenging activity toward peroxyl radicals than catechins, and

this was postulated to be due to the presence of interflavanoid linkage, increasing the electron delocalization capacity of the phenyl radical. In the present study, these structures were not compared separately. However, no difference was found in the free radical scavenging ability between bilberry fractions containing either catechins with one B-type dimer or a mixture of A-type and B-type procyanidins. In the case of other berries, the differences between the fractions reflect mostly the total contents of catechins and procyanidins in these fractions. Lingonberry and cranberry catechins and procyanidins were more efficient as radical scavengers than bog whortleberry and bilberry catechins and procyanidins. The differences between the berries are not only due to the total contents of catechol units as in the case of bog whortleberry procyanidins but are also due to the composition of catechins and procyanidins present in the fractions. Earlier Ho et al. (*34*) reported the strongest superoxide scavenging activity for the A-type dimer among the six typical lingonberry procyanidins. Moreover, the sterical structures play an important role in the ability to scavenge free radicals. (+)-Catechin was more powerful in scavenging peroxyl radicals than (−)-epicatechin when grape seed phenolics were evaluated (*35*). Similar results were obtained concerning the ability of catechin epimers to scavenge the free radical generated from 2,2′-azobis(2-amidinopropane) (AAPH) and DPPH (*36*). Therefore, the dominance of (+)-catechin in lingonberry may partly explain its higher activity in the DPPH test compared to bilberry and bog whortleberry, in which (−)-epicatechin dominated.

In our previous study, the radical scavenging activities of bilberry and lingonberry anthocyanins were found to be 38% and 25%, respectively, at the concentration of 8.3 μg/mL (*22*), determined under similar conditions as in the present study. By direct comparison of the inhibition percentages, the *Vaccinium* berry catechins and procyanidins appear to be more efficient radical scavengers than the respective anthocyanins.

Chain polymerization of catechin monomers to procyanidin trimers decreased the ability to prevent free radical damage in the lipid system and increased the antioxidant activity in the aqueous phase (*37*). Lotito et al. (*38*) reported partly contrary results, which were explained by the conditions used in the liposome oxidation test. In the present study, no difference was observed between monomers, dimers, and trimers in their radical scavenging activities or antioxidant activities toward the oxidation of methyl linoleate emulsion. When prevention of liposome oxidation was assessed, lingonberry procyanidins were reported to be the most active berry phenolics toward both lipids and proteins (*14*). Dimeric procyanidins B1 and B2 were reported by Viljanen et al. (*39*) to be better antioxidants against liposome oxidation compared to anthocyanins, since they contain several hydroxyl groups. The antioxidant effect against the oxidation of methyl linoleate emulsion achieved by *Vaccinium* berry procyanidins is comparable to the activity of anthocyanins isolated from bilberries and lingonberries (inhibition 60−90% at the levels 100 and 500 ppm) (*22*).

The number of catechol units in the reaction mixture was found to positively correlate with the ability of catechins and procyanidins to protect against oxidation of LDL, independently of monomer or chain structure (*40*). In the present study, lingonberry, bilberry, and cranberry catechins and low molecular weight procyanidins inhibited LDL oxidation equally at the higher concentration. In fact, bog whortleberry catechins were excellent antioxidants toward the oxidation of human LDL at both tested concentrations. Cranberry and bilberry fractions promoted oxidation at the lower concentration level (2.5 μg/mL) similarly to the low content of catechol units in bog whortleberry procyanidin fraction. These pro-oxidant effects may simply be due to the fact that the low content of catechol units cannot achieve the saturation point for the antioxidant effect in the LDL model. The present results for catechins and procyanidins are comparable to earlier findings on antioxidant and pro-oxidant properties of berry anthocyanins toward human LDL (*22*). The differences between *Vaccinium* berries in the composition of catechins and procyanidins contributed to their antioxidant and pro-oxidant effects. In summary, radical scavenging and antioxidant activities of *Vaccinium* fractions were not only dose dependent but also attributable to the composition of catechins and procyanidins in the fractions and in the respective berries. *Vaccinium* berries rich in phenolic compounds including catechins and procyanidins represent antioxidant sources that may have potential for inducing beneficial effects on human health.

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