JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY



# Anthocyanin Composition and Antioxidant Activity of the Crowberry (*Empetrum nigrum*) and Other Berries

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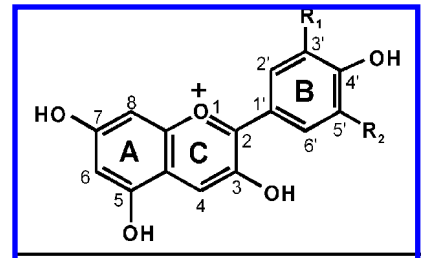
The anthocyanin composition and antioxidant activity of the crowberry (*Empetrum nigrum*) were studied. High-performance liquid chromatography (HPLC) combined with a diode array detector and electrospray ionization mass spectrometry were used for identification and quantification of individual anthocyanins. Freeze-dried crowberry powder was extracted with 80% methanol containing 0.5% acetic acid and subjected to HPLC. Thirteen kinds of anthocyanins were identified. The major anthocyanins were cyanidin-3-galactoside and delphinidin-3-galactoside, at 8.04 and 8.62 mg/g extract, respectively. The HPLC profile of crowberry extract was similar to bilberry and blueberry. The total content of anthocyanins in crowberry was 41.8 mg/g extract, higher than the other nine major berry species (2.5−38.8 mg/g extract). The antioxidant activity was also evaluated using the 1,1-diphenyl-2-picrylhydrazyl and 2,2′-azinobis(3-ethybenzothiazoline-6-sulfonic acid) radical quenching assays and the ferric reducing activity power assy. Crowberry extract exerted the strongest antioxidant activity. In conclusion, individual anthocyanins in crowberry were identified and then quantified in this study. Additionally, crowberry is suggested to be associated with a reduction in the risk of developing chronic diseases because of its strong antioxidant activity.

**KEYWORDS: Crowberry; berry; anthocyanin; HPLC-ESI-MS/MS; antioxidant activity**

## INTRODUCTION

Anthocyanidins, which have a typical flavonoid structure (**Figure 1**), are mostly plant pigments responsible for red, blue, and purple colors and are widely distributed in colored fruits and vegetables, especially berries, as glycoside forms, named anthocyanins (*1–4*). Anthocyanidins and anthocyanins have been known to exhibit various biological effects, for example, antioxidant activity, anticarcinogenesis, apoptosis induction, antiobesity, antidiabetes, and prevention of DNA damage (*5–12*). Therefore, regular consumption of anthocyanin-rich foods has been considered to be associated with a reduction in the risk of developing chronic diseases (*13, 14*). To investigate this possibility, it is important to estimate the daily consumption of anthocyanins. The daily consumption of anthocyanins was recently reported to be 12.5 mg/day in the United States (*1*) and 82 mg/day in Finland (*15*). On the other hand, in an earlier paper, intake was estimated to far exceed this amount at 180−215 mg/day (*16*). Such huge differences in estimates of the daily intake of anthocyanins were ascribed to result from different food intake data (*1*). This indicates that more accurate data about anthocyanin content in our daily foods are important.




| | $R_1$ | $R_2$ |
|---|---|---|
| Cyanidin | OH | H |
| Delphinidin | OH | OH |
| Malvidin | $OCH_3$ | $OCH_3$ |
| Pelargonidin | H | H |
| Peonidin | $OCH_3$ | H |
| Petunidin | $OCH_3$ | OH |

**Figure 1.** Structures of anthocyanidins.

The distribution of anthocyanins has been well-studied in detail in some berries, for example, bilberry, blueberry, blackcurrant, and strawberry (*1, 17–20*). Recently, another edible berry, the crowberry (*Empetrum nigrum*), has attracted attention

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10.1021/jf800406v CCC: $40.75 © 2008 American Chemical Society
Published on Web 06/04/2008

**Table 1.** Berries Used in This Study

| English name | scientific name | contents (g/100 g fresh berry) of water[a] | extract[b] |
|---|---|---|---|
| bilberry | *Vaccinium myrtillus* | 85.4 | 5.7 |
| blackberry | *Rubus fruticosus* | 85.0 | 5.0 |
| blackcurrant | *Ribes nigrum* | 77.0 | 8.8 |
| blueberry | *Vaccinium* spp. | 85.4 | 6.7 |
| cranberry | *Vaccinium oxycoccus* | 84.5 | 6.6 |
| crowberry | *Empetrum nigrum* | 87.3 | 3.0 |
| mulberry | *Morus nigra* | 81.9 | 6.3 |
| raspberry | *Rubus idaeus* | 84.2 | 5.5 |
| redcurrant | *Ribes rubrum* | 83.4 | 6.6 |
| strawberry | *Fragaria* × *ananassa* | 88.4 | 5.2 |

[a] Calculated after freeze drying of fresh berry. [b] Remainder extracted by 80% methanol containing 0.5% acetic acid from freeze-dried berry power.

because of its putative benefits. Crowberry is commonly found in the northern hemisphere, especially in Finland (*21, 22*), and is consumed as a juice (*22*). The total antioxidant concentration in the crowberry was reported to be a little less than in the blackcurrant, similar to that of the blackberry, and greater than that of the blueberry and raspberry (*23*). The total content of flavonols, which is the total sum of quercetin, myricetin, and kaempferol, was higher than that of commonly consumed fruits or vegetables other than onion, kale, and broccoli (*24*). In addition, wines made of a mixture of crowberry and blackcurrants have slightly superior antioxidant activities as compared with red grape wines (*25*). The crowberry is also an excellent dietary source of anthocyanidins. The quantities are almost the same level as that in the bilberry, which contains the highest amount of anthocyanins of the berries (*2, 3*). This indicates that the crowberry might be a candidate as a daily foodstuff to protect the body from chronic diseases. However, there are no studies in detail about individual anthocyanins in the crowberry as far as we are concerned, although it is well-known that almost all anthocyanins in the berries exist as glycoside forms (*17*). In this study, we therefore identified and quantified individual anthocyanidin glycosides (anthocyanins) in crowberries using high-performance liquid chromatography (HPLC) combined with a diode array detector (DAD) and electrospray ionization mass spectrometer (ESI-MS) system. Additionally, their amounts and the antioxidant activity of the crowberry were compared with those of nine other famous kinds of berries: bilberry, blackberry, blackcurrant, cranberry, redcurrant, blueberry, mulberry, raspberry, and strawberry.

## MATERIALS AND METHODS

**Chemicals.** The standard anthocyanidins and anthocyanins, delphinidin, delphinidin-3-glucoside, cyanidin, cyanidin-3-galactoside, cyanidin-3-glucoside, cyanidin-3-rutinoside, petunidin, peonidin, peonidin-3-glucoside, pelargonidin, pelargonidin-3-glucoside, malvidin, malvidin-3-galactoside, and malvidin-3-glucoside, were obtained from Extrasynthese (Genay, France). Trifluoroacetic acid (TFA), 2,2′-azinobis(3-ethybenzothiazoline-6-sulfonic acid) (ABTS), 2,4,6-tripyridyl-1,3,5-triazine (TPTZ), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). *tert*-Butylhydroquinone (BHQ) was from Sigma Aldrich, Inc. (St. Louis, MO). All other reagents were of the highest grade available.

**Berries.** Ten kinds of fresh berries were used in this study. Crowberry (*Empetrum nigrum*) and bilberry (*Vaccinium myrtillus*) were kindly donated from Wakasa Seikatsu, Co., Ltd. (Kyoto, Japan). Blackberry (*Rubus fruticosus*), blackcurrant (*Ribes nigrum*), cranberry (*Vaccinium oxycoccus*), and redcurrant (*Ribes rubrum*) were obtained from Sicoly (St. Laurent d'Agny, France). Blueberry (*Vaccinium* spp.), mulberry (*Morus nigra*), and raspberry (*Rubus idaeus*) were from Life Foods Co., Ltd. (Tokyo, Japan), Sakurae Kuwatya Seisan Kumiai (Shimane, Japan), and Ocean Trading Co., Ltd. (Kyoto, Japan), respectively. Strawberry (*Fragaria* × *ananassa*) was obtained from a farmhouse in Shizuoka City (Shizuoka, Japan). Each fresh berry (100 g) was homogenized in liquid nitrogen and lyophilized using a freeze-dryer Eyela FD-5N (Tokyo Rikakikai, Co., Ltd., Tokyo, Japan). The freeze-dried powder was stored at 4 °C in a desiccator until analysis.

**Extraction of Anthocyanins.** The extraction of anthocyanins from berries was done according to the method reported by Sakakibara et al. with some modifications (*4*). Briefly, the stored powders (200 mg) were added to 8 mL of 80% methanol containing 0.5% acetic acid. The solution was allowed to stand in a sonicater for 1 min, and the supernatant was recovered by centrifugation at 4000 rpm for 10 min under 4 °C. After extraction three times, the supernatants were gathered and then dried with the freeze dryer. The dried extracts were kept at 4 °C in a desiccator with protection from light.

**HPLC-DAD.** Ten milligrams of berry extract was dissolved in 1 mL of 50% ethanol containing 0.5% BHQ and 0.5% TFA and filtered through a 0.45 $\mu$m membrane filter (Nacalai Tesque, Inc., Kyoto, Japan) according to the modified method reported by Sakakibara et al. 4. The HPLC system employed to analyze anthocyanins was a Jasco system control program HSS-1500 (Tokyo, Japan) equipped with Jasco-Borwin chromatography data station, pump PU-1580, autosampler AS-1559, column oven CO-1565, and DAD system MD-1510 for monitoring at all wavelengths from 200 to 600 nm. For the column, Capcell Pak ACR ($\phi$4.6 mm × 250 mm, S-5, 5 $\mu$m, Shiseido Co., Ltd., Tokyo, Japan) was used at 40 °C. Linear gradient elution was performed with solution A (0.5% TFA aqueous) and solution B (acetonitrile containing 0.1% TFA) delivered at a flow rate of 1.0 mL/min as follows: initially 92% of solution A; for the next 50 min, 85% A; for another 10 min, 70% A; for another 5 min, 40% A; and finally keep 40% A for 10 min. The injection volume for the extract was 10 $\mu$L.

**HPLC-ESI-MS/MS Analysis.** Another set of extracts dissolved in 1 mL of 50% ethanol containing 0.5% BHQ and 0.5% TFA was injected into the HPLC-ESI-MS/MS system to obtain more information about anthocyanins in berries. A portion of the filtrate (5 $\mu$L) was subjected to HPLC on a Capcell Pak ACR ($\phi$2.0 mm × 250 mm, S-5, 5 $\mu$m). The columns were maintained at 40 °C. Linear gradient elution was performed with solution A (10% formic acid) and solution B (acetonitrile) delivered at a flow rate of 0.2 mL/min as follows: initially 98% of solution A; for the next 10 min, 93% solution A; for another 25 min, 70% A; for another 5 min, 40% A; and finally 40% A for 10 min. The elution of anthocyanins was monitored at 520 nm (Nanospace SI-1 HPLC system, Shiseido, Tokyo) and then introduced into the ion-trap electrospray mass spectrometer equipped with ESI (LCQ, Thermo Fisher Scientific K.K., San Jose, CA). The mass spectrometer was operated in the positive ion mode in the range $m/z$ 100−1000 under the following conditions: detector voltage, 5.0 kV; capillary voltage, 10 V; and capillary temperature, 200 °C.

**DPPH Radical Quenching Assay.** The DPPH radical quenching assay was carried out according to the method reported by Blois (*26*). Each berry extract was dissolved in 50% ethanol at a concentration of a 2 mg/mL. The sample or 0.1 mg/mL trolox or solvent (100 $\mu$L) was added into 3 mL of 100 $\mu$M DPPH in ethanol, and absorbance at 517 nm was measured after 30 min at room temperature. The antioxidant activity of the berries was calculated as the DPPH radical quenching activity (%) as compared with the data using only DPPH.

**ABTS Radical Quenching Assay.** The ABTS radical was generated through a chemical oxidation reaction with potassium persulfate as described by Re et al. (*27*). Briefly, 100 mL of 7 mM ABTS solution and 50 mL of 7.35 mM potassium persulfate solution were mixed and left for 12 h at room temperature. The concentration of the ABTS radical solution was adjusted with ethanol to an absorbance at 734 nm from 0.80 to 0.90. The sample (2 mg/mL) or 0.1 mg/mL trolox or solvent (100 $\mu$L) was added into 3 mL of ABTS radical solution and incubated at room temperature for 5 min, and the absorbance at 734 nm was measured immediately. The percentage inhibition of the radical scavenging activity was calculated.

**Ferric Reducing Activity Power (FRAP) Assay.** The FRAP assay was carried out as described by Benzie and Strain (*28*) with a little modification. Briefly, FRAP reagent consisted of 10 mM TPTZ solution in 40 mM hydrochloric acid, 300 mM sodium acetate buffer (pH 3.6),

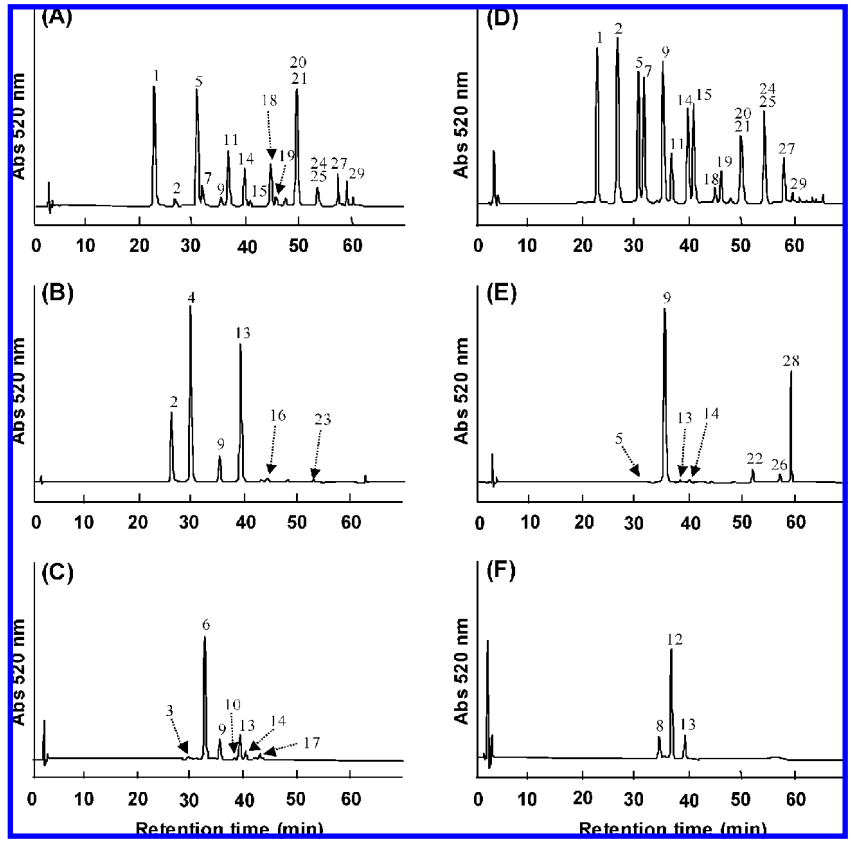


**Figure 2.** HPLC profiles of berry extracts at 520 nm. Each berry was extracted with 80% methanol containing 0.5% acetic acid as described in the Materials and Methods. (**A**) Crowberry, (**B**) blackcurrant, (**C**) raspberry, (**D**) bilberry, (**E**) blackberry, and (**F**) redcurrant. Peak numbers refer to **Table 2**.

**Table 2.** Identified Anthocyanins in Berries

| peak | RT (min)[a] | anthocyanins | $[M]^+$ (*m*/*z*) | fragment ions (*m*/*z*) |
|---|---|---|---|---|
| 1 | 23.1 | delphinidin-3-galactoside | 465 | 303 |
| 2 | 26.8 | delphinidin-3-glucoside | 465 | 303 |
| 3 | 29.4 | cyanidin-3-sophoroside | 611 | 449, 287 |
| 4 | 29.7 | delphinidin-3-rutinoside | 611 | 465, 303 |
| 5 | 31.1 | cyanidin-3-galactoside | 449 | 287 |
| 6 | 32.0 | cyanidin-3-sophoroside-5-rhamnoside | 757 | 611, 433, 287 |
| 7 | 32.1 | delphinidin-3-arabinoside | 435 | 303 |
| 8 | 35.0 | cyanidin3-sambubioside | 581 | 287 |
| 9 | 35.6 | cyanidin-3-glucoside | 449 | 287 |
| 10 | 36.1 | cyanidin-3-sambubioside-5-rhamnoside | 727 | 581, 433, 287 |
| 11 | 37.1 | petunidin-3-galactoside | 479 | 317 |
| 12 | 38.0 | cyanidin-3-xylosylrutinoside | 727 | 581, 287 |
| 13 | 39.3 | cyanidin-3-rutinoside | 595 | 449, 287 |
| 14 | 40.4 | cyanidin-3-arabinoside | 419 | 287 |
| 15 | 41.4 | petunidin-3-glucoside | 479 | 317 |
| 16 | 43.3 | petunidin-3-rutinoside | 625 | 479, 317 |
| 17 | 44.8 | pelargonidin-3-rutinoside | 578 | 431, 271 |
| 18 | 45.3 | peonidin-3-galactoside | 463 | 301 |
| 19 | 46.4 | petunidin-3-arabinoside | 449 | 317 |
| 20 | 50.2 | peonidin-3-glucoside | 463 | 301 |
| 21 | 50.2 | malvidin-3-galactoside | 493 | 331 |
| 22 | 51.9 | cyanidin-3-xyloside | 419 | 287 |
| 23 | 53.7 | peonidin-3-rutinoside | 609 | 463, 301 |
| 24 | 54.7 | peonidin-3-arabinoside | 433 | 301 |
| 25 | 54.7 | malvidin-3-glucoside | 493 | 331 |
| 26 | 57.1 | cyanidin-3-(6″-malonoyl)glucoside | 535 | 449, 287 |
| 27 | 58.3 | malvidin-3-arabinoside | 463 | 331 |
| 28 | 59.1 | cyanidin-3-dioxaloylglucoside | 593 | 287 |

[a] Retention time of HPLC.

and 20 mM ferric chloride(III) solution at the ratio of 10:1:1 (v/v/v), respectively. The sample (2 mg/mL) or solvent (100 $\mu$L) was added into 3 mL of FRAP reagent and incubated at room temperature for 3 min, and the absorbance at 593 nm was measured immediately. The results were calculated as mg trolox equivalent/mL.

## RESULTS AND DISCUSSION

The berry fruits used in this study are summarized in **Table 1**. All berries were freeze-dried, and the water content was calculated. The freeze-dried powders were then extracted with 80% methanol containing 0.5% acetic acid to obtain the anthocyanin fraction.

**Identification and Calculation of Anthocyanins in Berries.** Ten kinds of berry were analyzed for individual anthocyanin content. **Figure 2** shows the typical HPLC chromatogram at 520 nm of the six berries including the crowberry. The retention times and spectra were compared with that of commercially available anthocyanidins and anthocyanins. The identification of the peaks, which had a spectrum typical of anthocyanins but retention times not consistent with standard compounds, was based on HPLC-ESI-MS/MS analysis, and the identity of the anthocyanins was compared to the published data in earlier studies (*1, 17–20, 29*). The anthocyanins identified in the 10 kinds of berries are summarized in **Table 2**. The identified peaks with no comparative standard anthocyanin were determined using calibration curves for commercially available anthocyanins, which had the same aglycon structures. For example, delphinidin-3-glucoside was used for calculation of delphinidin-3-arabinoside content. Peaks 20 and 21 in the **Figure 2** were identified as peonidin-3-glucoside and malvidin-3-galactoside, respectively. However, these two peaks had almost the same retention times and spectra under our HPLC conditions. Therefore, when the peak, which eluted at 50.2 min and had a typical anthocyanin spectrum, was obtained in the HPLC analysis, it was determined to be peonidin-3-glucoside/malvidin-3-galactoside and calculated using the calibration curve of peonidin-3-glucoside. Peaks 24 and 25 also gave almost the same retention time. Bilberry is one of the best recognized

**Table 3.** Anthocyanin Content in Fresh Berries

| berries | anthocynins | mg/g extract[a] |
|---|---|---|
| bilberry | cyanidin-3-galactoside | 3.70 |
| | cyanidin-3-glucoside | 4.05 |
| | cyanidin-3-arabinoside | 2.54 |
| | delphinidin-3-galactoside | 4.58 |
| | delphinidin-3-glucoside | 4.73 |
| | delphinidin-3-arabinoside | 3.53 |
| | peonidin-3-galactoside | 0.46 |
| | petunidin-3-halactoside | 1.52 |
| | petunidin-3-glucoside | 2.94 |
| | petunidin-3-arabinoside | 0.84 |
| | malvidin-3-arabinoside | 0.81 |
| | peonidin-3-glucoside/malvidin-3-galactoside | 3.48 |
| | peonidin-3-arabinoside/malvidin-3-glucoside | 3.62 |
| blackberry | cyanidin-3-glucoside | 7.17 |
| | cyanidin-3-rutinoside | 0.06 |
| | cyanidin-3-arabinoside | 0.05 |
| | cyanidin-3-xyloside | 0.47 |
| | cyanidin-3-(6-malonoyl)glucoside | 0.30 |
| | cyanidin-3-dioxaloylglucoside | 2.05 |
| blackcurrant | cyanidin-3-glucoside | 1.10 |
| | cyanidin-3-rutinoside | 7.08 |
| | delphinidin-3-glucoside | 2.94 |
| | delphinidin-3-rutinoside | 9.79 |
| | peonidin-3-rutinoside | 0.11 |
| | petunidin-3-rutinoside | 0.18 |
| blueberry | cyanidin-3-galactoside | 0.28 |
| | cyanidin-3-glucoside | 0.04 |
| | cyanidin-3-arabinoside | 0.12 |
| | delphinidin-3-galactoside | 1.37 |
| | delphinisin-3-glucoside | 0.13 |
| | delphinidin-3-arabinoside | 0.74 |
| | peonidin-3-galactoside | 0.15 |
| | petunidin-3-galactoside | 1.07 |
| | petunidin-3-glucoside | 0.11 |
| | petunidin-3-arabinoside | 0.46 |
| | marlidin-3-arabinoside | 1.75 |
| | peonidin-3-glucoside/malvidin-3-galactoside | 3.65 |
| | peonidin-3-arabinoside/malvidin-3-glucoside | 0.43 |
| cranberry | cyanidin-3-galactoside | 3.86 |
| | cyanidin-3-glucoside | 0.27 |
| | cyanidin-3-arabinodside | 0.62 |
| | peonidin-3-glucoside | 0.05 |
| crowberry | cyanidin-3-galactoside | 8.04 |
| | cyanidin-3-glucoside | 0.55 |
| | cyanidin-3-arabinoside | 2.34 |
| | delphinidin-3-galactoside | 8.62 |
| | delphinidin-3-glucoside | 0.43 |
| | delphinidin-3-arabinoside | 1.36 |
| | peonidin-3-galactoside | 2.57 |
| | petunidin-3-galactoside | 3.81 |
| | petunidin-3-glucoside | 0.24 |
| | petunidin-3-arabinoside | 0.52 |
| | malvidin-3-arabinoside | 1.41 |
| | peonidin-3-glucoside/malvidin-3-galactoside | 10.37 |
| | peonidin-3-arabinoside/malvidin-3-glucoside | 1.55 |
| mulberry | cyanidin-3-halavtoside | 0.03 |
| | cyanidin-3-glucoside | 9.61 |
| | cyanidin-3-rutinoside | 4.22 |
| | pelargonidin-3-glucoside | 1.74 |
| | pelargonidin-3-rutinoside | 0.54 |
| raspberry | cyanidin-3-sophoroside | 6.73 |
| | cyanidin-3-sophoroside-5-rhamnoside | 1.21 |
| | cyanidin-3-glucoside | 0.98 |
| | cyanidin-3-sambubioside-5-rhamnoside | 0.46 |
| | cyanidin-3-rutinoside | 0.30 |
| | pelargonidin-3-glucoside | 0.66 |
| redcurrant | cyanidin-3-sambubioside | 0.31 |
| | cyanidin-3-xylosylrutinoside | 1.82 |
| | cyanidin-3-rutinoside | 0.36 |
| strawberry | cyanidin-3-glucoside | 0.09 |
| | pelargonidin-3-glucoside | 5.07 |

[a] Values are expressed as mean of triplicate analyses for each sample.

**Table 4.** Antioxidant Potency of Berry Extracts

| | | antioxidant potencies[b,c] by | | |
|---|---|---|---|---|
| berries | total anthocyanins[a] | DPPH | ABTS | FRAP |
| bilberry | 38.8 | 61 ± 0.3 | 38 ± 0.3 | 193 ± 1.1 |
| blackberry | 10.1 | 51 ± 0.4 | 26 ± 0.4 | 124 ± 1.0 |
| blackcurrant | 21.2 | 52 ± 1.0 | 31 ± 0.1 | 131 ± 4.3 |
| blueberry | 10.2 | 28 ± 0.6 | 14 ± 0.1 | 56 ± 2.3 |
| cranberry | 4.8 | 36 ± 0.4 | 23 ± 0.3 | 94 ± 1.3 |
| crowberry | 41.8 | 90 ± 0.4 | 64 ± 0.7 | 317 ± 1.9 |
| mulberry | 16.1 | 40 ± 0.4 | 17 ± 0.2 | 89 ± 0.3 |
| raspberry | 10.3 | 46 ± 0.5 | 23 ± 0.2 | 94 ± 1.7 |
| redcurrant | 2.5 | 50 ± 0.4 | 27 ± 0.8 | 95 ± 0.2 |
| strawberry | 5.2 | 25 ± 0.1 | 11 ± 0.2 | 40 ± 0.3 |
| trolox | | 47 ± 0.4 | 20 ± 0.1 | |

[a] mg/g extract. [b] Berry extracts (2 mg/mL) and trolox (0.1 mg/mL) were used for each method. [c] Antioxidant potencies were indicated as % of radical quenching activity (DPPH and ABTS) and mg trolox equivalent/mL (FRAP) as described in the Materials and Methods.

berries in the world, and the anthocyanin content has been well-studied. In this study, 13 kinds of anthocyanin were detected in the bilberry (**Table 3**). Cyanidin and delphinidin were major aglycon structures. These results were similar to previous reports (*17*). Additionally, as shown in **Table 4**, the total anthocyanin content in the bilberry was 38.8 mg/g extract (ca. 566 mg/100 g fresh berry), and this amount was almost the same as reported by Maatta-Riihinen et al. (*2*) and Koponen et al. (*3*), indicating that the method used in this study was quite reliable for extraction, identification, and quantification of anthocyanins in berries. The results obtained from other berries, for example, blackcurrant, blueberry, and strawberry, also support the validity of our method (*2, 17, 18, 20, 29*).

**Anthocyanins in Crowberry.** As shown in **Table 3**, 13 kinds of anthocyanins were identified in crowberry, and all were based on the five common anthocyanidin structures (**Figure 1**). The aglycons themselves were under the detection limit in this study. Major anthocyanins were cyanidin-3-galactoside, delphinidin-3-galactoside, and peonidin-3-glucoside/malvidin-3-galactoside, and their quantities were 8.04, 8.62, and 10.37 mg/g extract, respectively. These patterns were quite similar to those of the bilberry and blueberry and differed extensively from other berries. In this study, we reported first identification and quantification of individual anthocyanins in the crowberry. We consider that the crowberry and its processing into foods and drinks like wine provide a good source of anthocyanins in our daily life.

**Antioxidant Activities.** The antioxidant activity of flavonoids has been extensively related to their chemical structures. For example, numbers and/or positions of the hydroxyl group on the B ring are of great importance (*30*). The superoxide anion radical scavenging activity and the inhibitory effect on hydrogen peroxide-induced lipid peroxidation have been reported to be stronger with more hydroxyl groups on the B ring, in the order delphinidin (3′,4′,5′-OH) > cyanidin (3′,4′-OH) > peralgonidin (4′-OH) (*30*). For this reason, the types of aglycon structures are important for antioxidant activity in the berries, in addition to the total amount of anthocyanin. The total content of anthocyanins in the crowberry (41.8 mg/g extract) was higher than in the other berries used in this study (2.5−38.8 mg/g extract) (**Table 4**). Additionally, major aglycons in the crowberry were delphinidin and cyanidin, similar to the bilberry and blackberry (**Table 3**). The antioxidant activities of the berries were evaluated using three types of ordinary antioxidant evaluation methods, the DPPH and ABTS radical quenching assay and the FRAP assay (**Table 4**). Trolox at 3.2 $\mu$g/mL in

the reaction mixture (ca. 12.9 $\mu$M) quenched DPPH and ABTS radicals by 47 and 20%, respectively, comparable to previous results (*31*). Antioxidant activities in the berries were compared at the same concentration of 65 $\mu$g extract/mL of reaction mixture (**Table 4**). In both radical scavenging methods, the crowberry showed the strongest antioxidant activity. The crowberry also had the highest activity according to the FRAP assay, suggesting that the crowberry is one of the most powerful antioxidant berries. On the other hand, the antioxidant potency is not well-matched to the contents of total anthocyanins in the berries. These results suggest that different amounts of other antioxidants, for example, ascorbic acid or flavonols such as quercetin and kaempferol (*24, 32*), in the berries contributed to their antioxidant properties, although major antioxidants in the berries might be anthocyanins.

Berries have been reported to have a broad spectrum of biomedical uses, such as in cardiovascular disorders, advancing age-induced oxidative stress, inflammatory disorders, and diverse degenerative diseases (*13, 14*). The active ingredients for these beneficial effects in the berries are speculated to be anthocyanins (*5–10*). Recently, consumption of the berries has been suggested to be beneficial in reversing the course of neuronal and behavioral aging, perhaps due to their antioxidant properties (*33, 34*). This study demonstrated that the crowberry is an anthocyanin-rich berry and contains high quantities of delphinidin glycosides, which have the strongest antioxidant activity of the anthocyanins. In addition, anthocyanins have been suggested to improve visual function. Interestingly, Matsumoto et al. reported that cyanidin glycosides stimulate the regeneration of rhodopsin, but delphinidin glycosides do not have this effect (*35*). The crowberry also contains high amounts of cyanidin glycosides, indicating that consumption of the crowberry may be considered to be associated with a reduction in the risk of developing chronic diseases including visual impairment. However, further studies are needed to investigate these hypotheses. The present study, therefore, warrants further examination to explore the beneficial functions of the crowberry, including the effect on visual function.

## ACKNOWLEDGMENT

We thank for Wakasa Seikatsu, Co., Ltd. (Kyoto, Japan) for kindly supplying us the fresh crowberry and bilberry. This study was performed as part of the Global COE program at the University of Shizuoka.

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**Received for review February 9, 2008. Revised manuscript received April 21, 2008. Accepted April 22, 2008.**

JF800406V