JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

# Identification of Cyanidin 3-*O*-$\beta$-(6″-(3-Hydroxy-3-methylglutaroyl)glucoside) and Other Anthocyanins from Wild and Cultivated Blackberries

Monica Jordheim,* Kjersti Hasle Enerstvedt, and Øyvind M. Andersen

Department of Chemistry, University of Bergen, Allégt. 41, 5007 Bergen, Norway

**ABSTRACT:** Anthocyanins from blackberries are natural dietary pigments. The aim of this study was to investigate the occurrence of anthocyanins in fruits of wild Norwegian blackberries and three blackberry (*Rubus fruticosus* L.) cultivars and to report the complete identification of cyanidin 3-*O*-$\beta$-(6″-(3-hydroxy-3-methylglutaroyl)glucopyranoside), **5**. This new pigment is most probably the same pigment that has previously been reported to occur in various blackberry samples as cyanidin 3-dioxalylglucoside. All of the examined blackberry samples contained in similar relative proportions the 3-glucoside (**1**), 3-rutinoside (**2**), 3-xyloside (**3**), and 3-*O*-$\beta$-(6″-malonylglucoside) (**4**) of cyanidin and **5**. The absolute amounts of **1**−**5** in the wild Norwegian blackberries were 249, 18, 10, 24, and 22 mg of cyanidin 3-glucoside equivalents/100 g of fresh weight, respectively.

**KEYWORDS:** blackberries, *Rubus fruticosus* L., anthocyanin pigments, cyanidin 3-*O*-$\beta$-(6″-(3-hydroxy-3-methylglutaroyl)-glucoside), quantitative content, NMR

## INTRODUCTION

In addition to their roles as secondary metabolites in many flowers, fruits, leaves, etc., anthocyanins have gained attention as functional pigments for coloring of food. Interests in anthocyanins have intensified in recent years because of their possible health benefits as dietary antioxidants. Over 650 naturally occurring anthocyanins have hitherto been reported.[1] They occur primarily as glycosides of their respective aglycones (anthocyanidins), and more than half of them are acylated with aromatic and/or aliphatic acyl moieties. The kind of structure influences the occurrence of each anthocyanin in the various equilibrium forms, and both structure diversity and differences in equilibrium form distribution are reflected as differences in terms of stability, bioavailability, and potential health effects.[2,3] In this context it is well-known that anthocyanins acylated with aromatic acyl groups have different properties from their nonacylated analogues. Compared to flowers, the anthocyanin structures found in commonly eaten fruits and berries are rather simple in nature, and just a very few of them are acylated.[2]

Blackberries are a rich source of anthocyanins and are reported to have significant antioxidant capacity.[4−8] Several authors have characterized individual anthocyanins of various blackberry species and cultivars.[9] There seems to be some discrepancy with respect to the exact nature of the individual anthocyanins, however; one or more of the 3-glucoside, 3-rutinoside, and 3-xyloside (or 3-arabinoside) of cyanidin have been reported by most authors.[9−15] In addition, cyanidin 3-(6″-malonylglucoside) and a second acylated cyanidin derivative have been reported in more recent studies. Rommel et al. have shown that the acylated cyanidin derivatives of blackberry wine and juice had greater stability than nonacylated cyanidin derivatives during fermentation and were also resistant to degradation by commercial pectinase enzyme preparations.[16] Sapers et al. showed that the less polar of the two acylated anthocyanins was the main pigment (51%) in unripe blackberries of the cultivar Hull Thornless.[10] This acylated pigment was identified by Stintzing et al. to be cyanidin 3-dioxalylglucoside.[12] Later, several studies of individual anthocyanins from various blackberry samples have reported the same anthocyanin;[11,17−21] however, these identifications were mainly based on low-resolution MS and HPLC data. Our main objective is to report the structure of cyanidin 3-*O*-$\beta$-(6″-(3-hydroxy-3-methylglutaroyl)glucopyranoside), which is a new pigment with an uncommon acyl moiety. This pigment is most probably the same pigment that has previously been reported to occur in various blackberry samples as cyanidin 3-dioxalylglucoside.[11,12,17−21] This study investigates further the occurrence of individual anthocyanins in fruits of wild Norwegian blackberries and three blackberry (*Rubus fruticosus* L.) cultivars.

## MATERIALS AND METHODS

**Plant Material.** Fruits of Norwegian wild blackberry (*R. fruticosus* L.) and the cultivar Thornless Evergreen were collected at Foldøy (Rogaland, Norway). Fruits of cultivar Tupi (originally from Mexico) and cultivar Loch Ness (grown in the United Kingdom) were bought in a local London (U.K.) food store.

**Isolation of Pigments.** About 875 g of Norwegian wild blackberry fruits was extracted with acidified (0.5% trifluoroacetic acid (TFA), Merck, Darmstadt, Germany) methanol. The filtered extract was concentrated under reduced pressure at 27 °C and applied to an Amberlite XAD-7 column (70 × 5 cm, Sigma-Aldrich, Steinheim, Germany). The anthocyanins adsorbed to the column were washed with water and eluted from the column with methanol containing 0.5% TFA. The concentrated anthocyanin extract was applied to a Sephadex LH-20 column (10 × 80 cm, Amersham Biosciences, Uppsala, Sweden) using $H_2O$/MeOH/TFA (79.5:20:0.5, v/v/v) as eluent. For further isolation

Received: April 16, 2011
Revised: June 1, 2011
Accepted: June 3, 2011
Published: June 03, 2011





dx.doi.org/10.1021/jf201522b | *J. Agric. Food Chem.* 2011, 59, 7436–7440

**Table 1. Retentions Times (HPLC), High-Resolution Electrospray MS, and Quantitative Data for Anthocyanins Identified in Wild Norwegian Blackberries**

| | anthocyanin | $t_R$ (min) | $[M]^+$ obsd ($m/z$) | $[F]^+$ obsd ($m/z$) | $[M]^+$ calcd ($m/z$) | molecular formula | quantitative amount[a] |
|---|---|---|---|---|---|---|---|
| **1** | cyanidin 3-glucoside[b] | 23.2 | 449.1084[b] | 287 | 449.1084 | $C_{21}H_{21}O_{11}^+$ | 249.0 ± 8.0 |
| **2** | cyanidin 3-rutinoside | 24.1 | 559.1673 | 287 | 595.1663 | $C_{27}H_{31}O_{15}^+$ | 18.3 ± 0.2 |
| **3** | cyanidin 3-xyloside | 29.2 | 419.0987 | 287 | 419.0978 | $C_{20}H_{19}O_{10}^+$ | 10.1 ± 0.3 |
| **4** | cyanidin 3-(6″-malonylglucoside) | 29.8 | 535.1106 | 287 | 535.1088 | $C_{24}H_{23}O_{14}^+$ | 24.3 ± 0.2 |
| **5** | cyanidin 3-(6″-(3-hydroxy-3-methylglutaroyl)glucoside) | 30.4 | 593.1518 | 287 | 593.1507 | $C_{27}H_{29}O_{15}^+$ | 21.6 ± 0.4 |

[a] mg cyanidin 3-glucoside equivalents/100 g FW. [b] Cyanidin 3-glucoside ($m/z$ 449.1084, calculated) was used as internal reference for MS data.

by preparative HPLC, Sephadex LH-20 fractions with similar qualitative anthocyanin contents (revealed by analytical HPLC) were combined.

**Preparative HPLC System.** The preparative HPLC system used a Gilson 321 pump equipped with an Ultimate 3000 variable wavelength detector, a 25 × 2.2 cm (10 μm) Econosphere C18 column (Grace, Deerfield, IL), and the solvents (A) water (0.5% TFA) and (B) acetonitrile (0.5% TFA). The elution profile for HPLC consisted of initial conditions with 90% A and 10% B followed by linear gradient elution for the next 10 min to 14% B, isocratic elution (10−14 min), and the subsequent linear gradient conditions: 14−18 min (to 16% B), 18−22 min (to 18% B), 22−26 min (to 23% B), 26-31 min (to 28% B), and 31−32 min (to 40% B), with isocratic elution at 32−40 min (40% B) and a final linear gradient elution at 43−46 min (to 10% B). The flow rate was 15 mL/min, and aliquots of 250 μL were injected.

**Analytical HPLC System.** The Agilent 1100 HPLC system was equipped with a HP 1050 diode array detector and a 200 × 4.6 mm inside diameter, 5 μm ODS Hypersil column (Supelco, Bellefonte, PA). Two solvents, (A) water (0.5% TFA) and (B) acetonitrile (0.5% TFA), were used for elution. The elution profile for HPLC consisted of initial conditions with 90% A and 10% B followed by a linear gradient elution for the next 10 min to 14% B, isocratic elution (10−14 min), and the subsequent linear gradient conditions: 14−18 min (to 16% B), 18−22 min (to 18% B), 22−26 min (to 23% B), 26−31 min (to 28% B), and 31−32 min (to 40% B), with isocratic elution at 32−40 min (40% B) and a final linear gradient elution at 43−46 min (to 10% B). The flow rate was 1.0 mL/min, and aliquots of 15 μL were injected with an Agilent 1100 series microautosampler. The UV−vis absorption spectra were recorded online during HPLC analysis over the wavelength range of 240−600 nm in steps of 2 nm.

**NMR Spectroscopy.** One-dimensional $^1$H, compensated attached proton test (CAPT), 2D heteronuclear single quantum coherence ($^1$H−$^{13}$C HSQC), heteronuclear multiple bond correlation ($^1$H−$^{13}$C HMBC), 2D correlation spectroscopy ($^1$H−$^1$H COSY), and 2D total correlation spectroscopy ($^1$H−$^1$H TOCSY) were obtained on a Bruker 600 MHz instrument equipped with a cryogenic probe. Sample temperatures were stabilized at 298 K. The deuteriomethyl $^{13}$C signal and the residual $^1$H signal of the solvent ($CF_3COOD$−$CD_3OD$; 5:95, v/v) were used as secondary references ($\delta$ 49.0 and 3.40 from TMS, respectively).

**LC-MS.** High-resolution LC-MS ($ESI^+$/TOF) spectra were recorded using a JMS-T100LC instrument with an AcuTOF LP mass separator. The gradient used was identical to the one described for the analytical HPLC system with one exception; TFA was replaced with 0.5% formic acid (HCOOH) in both solvents A (water) and B (acetonitrile). A 100 mm × 2.0 mm internal diameter, 3.0 μm Develosil C18 column (Phenomenex, Torrance, CA) was used for separation. Cyanidin 3-glucoside ($m/z$ 449.1084, calculated) was used as internal reference.

**Quantitative Determination.** Five grams of the wild blackberries was weighed and placed into a 15 mL screw-cap glass and extracted with 5 mL of acidified methanol (0.5% TFA) for 40 min in a refrigerator (4 °C). The extract was removed and stored in a sealed glass tube in a refrigerator. Extraction was repeated twice, and the combined extracts were transferred into a volumetric flask to determine the total volume followed by HPLC analysis. Five replicate samples were made. Prior to injection, the solutions were filtered through a 0.45 μm Millipore membrane filter.

The quantitative amounts of **1**−**5** were determined from a HPLC calibration curve of pure (>95%, determined by HPLC-DAD/NMR standardization) cyanidin 3-glucoside isolated from wild Norwegian blackberries,[22] without taking into account the variation of molar absorption coefficients for individual pigments. The results are presented as milligrams of cyanidin 3-glucoside equivalents per 100 g of fresh weight (FW) (Table 1). The relative quantitative amounts of anthocyanin **1**−**5** (Table 3), found in the three different blackberry cultivars were based on peak areas in analytical HPLC profiles. Five replicates of each cultivar were analyzed.

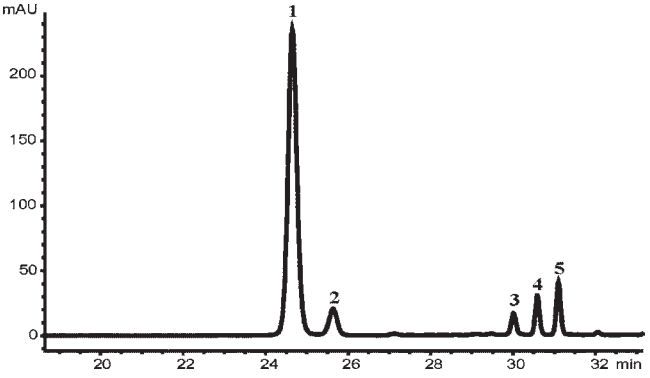


**Figure 1.** HPLC chromatogram of anthocyanins **1**−**5** in an extract of Norwegian wild blackberries detected at 520 ± 20 nm.

## RESULTS AND DISCUSSION

**Anthocyanins in Wild Norwegian Blackberries.** The HPLC profile (Figure 1) of the acidified methanolic extract of the wild blackberries detected at 520 ± 20 nm revealed one major (**1**) and four minor anthocyanins, **2**−**5** (Figure 2). The individual anthocyanins were isolated by Sephadex LH-20 chromatography and preparative HPLC after purification of the concentrated extract by Amberlite XAD-7 chromatography. The structures of the known pigments **1**−**4** were elucidated to be cyanidin 3-glucoside (**1**), cyanidin 3-rutinoside (**2**), cyanidin 3-xyloside (**3**), and cyanidin 3-*O*-β-(6″-malonylglucoside) (**4**), respectively, on the basis of high-resolution MS data (Table 1), NMR

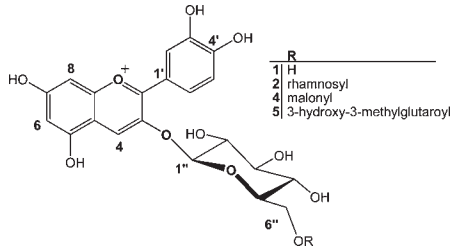


**Figure 2.** Structure of pigments **1**, **2**, **4**, and **5**. Pigment **3** is also a cyanidin monoglycoside, but with a xylosyl in the 3-OH position of the anthocyanidin.

spectroscopy, and online HPLC. The absolute amounts of **1**−**5** in the wild blackberries were found to be 249, 18, 10, 24, and 22 mg/100 g fresh weight, respectively (Table 1).

**Structure Elucidation of Pigment 5.** The $^{1}$H NMR spectrum of **5** showed six main proton signals in the aromatic region. The signals at $\delta$ 8.36 (dd, $J$ = 2.3, 8.7 Hz, H-6′), $\delta$ 8.12 (d, $J$ = 2.3 Hz, H-2′), and $\delta$ 7.11 (d, $J$ = 8.7 Hz, H-5′) coupled in a AMX spin system and at $\delta$ 9.05 (d, $J$ = 0.8 Hz, H-4), $\delta$ 6.99 (dd, $J$ = 0.8, 2.0 Hz, H-8), and $\delta$ 6.76 (d, $J$ = 2.0 Hz, H-6) in another AMX spin system were in accordance with the aglycone cyanidin (Table 2). The sugar region indicated the presence of one sugar unit including the anomeric proton (H-1″) at $\delta$ 5.38. The chemical shift values of the other sugar protons were identified from the cross-peaks at $\delta$ 5.38/3.76 (H-1″/H-2″), $\delta$ 3.76/3.63 (H-2″/H-3″), $\delta$ 3.63/3.53 (H-3″/H-4″), $\delta$ 3.53/3.86 (H-4″/H5″), $\delta$ 3.86/4.60 (H-5″/H-6a″), and $\delta$ 3.86/4.29 (H-5″/H-6b″) in the DQF-COSY spectrum. The corresponding sugar $^{13}$C resonances were identified from the cross-peaks in the HSQC spectrum, in accordance with $\beta$-glucopyranosyl (Table 2). The HMBC spectrum of **5** showed a cross-peak at $\delta$ 5.38/145.53 (H1″/C3), which confirmed the linkage between the aglycone and sugar unit to be at the cyanidin 3-hydroxyl.

The $^{1}$H and $^{13}$C signals of the acyl unit were assigned by interpretation of $^{1}$H NMR, DQF-COSY, TOCSY, HSQC, HMBC, and CAPT NMR spectra of **5** (Table 2; Figures 3 and 4) combined with high-resolution MS data (Table 1). The downfield NMR shifts of H-6a″ ($\delta$ 4.60) and H-6b″ ($\delta$ 4.29) of the glucosyl indicated the presence of acyl substitution, and both protons showed in the HMBC spectrum long-range cross-peaks with a carbonyl carbon at $\delta$ 172.45 (C-1‴). This carbonyl carbon showed in the same spectrum couplings with two proton signals at $\delta$ 2.80 and 2.71 belonging to a $CH_2$ group (H-2‴) (Figure 3). Another carbonyl carbon (C-5‴) showed similar long-range couplings to two proton signals at $\delta$ 2.68 and 2.62 belonging to a second $CH_2$ group (H-4‴) (Figure 3). The protons of these two methylene groups showed cross-peaks to the same methyl carbon (C-6‴) at $\delta$ 27.19 and a quaternary carbon (C-3‴) at $\delta$ 70.71. The relative downfield shift of this quaternary carbon indicated the attachment of an electronegative group such as a hydroxyl group. NMR correlations in the HMBC spectrum important for identification of the acyl moiety (3-hydroxy-3-methylglutaroyl) are presented in Figure 4. The high-resolution MS data (Table 1) were in accordance with cyanidin 3-*O*-$\beta$-(6″-(3-hydroxy-3-methylglutaroyl)glucopyranoside), which is a new pigment. The acyl moiety of **5**, 3-hydroxy-3-methylglutaroyl, has previously been found as part of an anthocyanin only in malvidin 3-*O*-$\beta$-(6″-(3-hydroxy-3-methylglutaroyl)glucoside) isolated from flowers of *Impatiens textori* Miq. (Balsaminaceae).[23]

**Table 2. $^{1}$H (600.13 MHz) and $^{13}$C (150.90 MHz) NMR Data for Cyanidin-3-*O*-$\beta$-(6″-(3-Hydroxy-3-methylglutaroyl)glucopyranoside) (5)[a]**

| | $^{1}$H $\delta$, $J$ (Hz) | $^{13}$C $\delta$ |
|---|---|---|
| 2 | | 164.71 |
| 3 | | 145.53 |
| 4 | 9.05 d, 0.8 | 136.92 |
| 5 | | 159.07 |
| 6 | 6.76 d, 2.0 | 103.46 |
| 7 | | 170.31 |
| 8 | 6.99 dd, 0.8, 2.0 | 95.27 |
| 9 | | 157.86 |
| 10 | | 113.07 |
| 1′ | | 121.75 |
| 2′ | 8.12 d, 2.3 | 118.47 |
| 3′ | | 147.31 |
| 4′ | | 155.93 |
| 5′ | 7.11 d, 8.7 | 117.41 |
| 6′ | 8.36 dd, 2.3, 8.7 | 128.46 |
| 1″ | 5.38 d, 7.8 | 103.64 |
| 2″ | 3.76 dd, 7.8, 9.7 | 74.68 |
| 3″ | 3.63 t, 9.7 | 73.69 |
| 4″ | 3.53 dd, 9.2, 9.7 | 71.28 |
| 5″ | 3.86 ddd, 2.1, 6.9, 9.2 | 75.98 |
| 6a″ | 4.60 dd, 2.1, 12.1 | 64.51 |
| 6b″ | 4.29 dd, 6.9, 12.1 | |
| 1‴ | | 172.45 |
| 2a‴ | 2.80 d, 14.7 | 46.08 |
| 2b‴ | 2.71 d, 14.7 | |
| 3‴ | | 70.71 |
| 4a‴ | 2.68 d, 15.3 | 45.43 |
| 4b‴ | 2.62 d, 15.3 | |
| 5‴ | | 174.39 |
| 6‴ (Me) | 1.37 | 27.19 |

[a] $^{13}$C NMR data are obtained from the $^{13}$C CAPT NMR spectra. s, singlet; d, doublet; dd, double doublet; ddd, double doublet of doublets; t, triplet; m, multiplet. Data were recorded in $CF_3CO_2D-CD_3OD$ (5:95, v/v) at 25 °C.

Several groups have reported cyanidin 3-dioxalylglucoside to occur in various blackberry species and cultivars.[11,12,17−21] The most comprehensive study among these with respect to structure elucidation of this pigment[12] has reported the structure of cyanidin 3-dioxalylglucoside to be tentative, without any detection of the intact dioxalyl moiety. When the various groups have included more experimental data than HPLC profiles as background for the characterization of cyanidin 3-dioxalylglucoside in blackberry samples, low-resolution MS data have been reported, which showed a molecular mass of $m/z$ 593, seemingly in agreement with cyanidin 3-dioxalylglucoside. The MS data on pigment **5** in the present study also give a molecular mass of $m/z$ 593. However, the high-resolution MS data for **5** ($m/z$ 593.1518) as well as the NMR data (Table 2) were in accordance with those of cyanidin 3-*O*-(6″-(3-hydroxy-3-methylglutaroyl)glucoside) (calcd $m/z$ 593.1507) and not in accordance with those of cyanidin 3-dioxalylglucoside (calcd $m/z$ 593.0779). Although we cannot disclose the possible coexistence of cyanidin 3-dioxalylglucoside and cyanidin 3-*O*-(6″-(3-hydroxy-3-methylglutaroyl)glucoside)

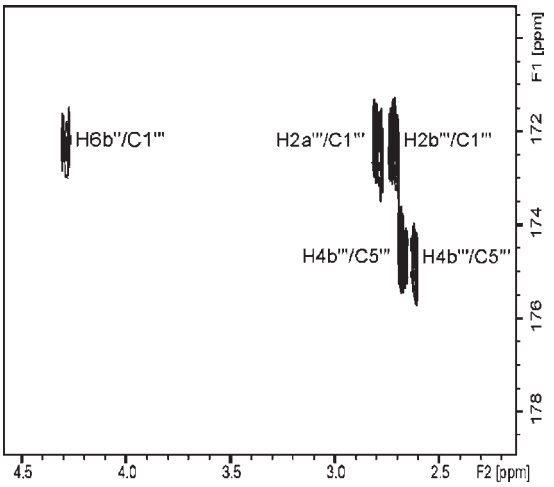


**Figure 3.** Expanded region of the HMBC spectrum of cyanidin 3-*O*-$\beta$-(6″-(3-hydroxy-3-methylglutaroyl)glucopyranoside) (**5**) in $CF_3CO_2D-CD_3OD$ (5:95, v/v) showing cross-peaks indicating the linkage between the acyl moiety and the sugar (H-6b″/C-1‴ ($^3J_{CH}$)) and neighborship between the two methylene groups and the carbon atoms of the two acyl groups of the 3-hydroxy-3-methylglutaroyl unit (H-2a‴/C-1‴ ($^2J_{CH}$), H-2b‴/C-1‴ ($^2J_{CH}$), H-4a‴/C-5‴ ($^2J_{CH}$), and H-4b‴/C-5‴ ($^2J_{CH}$)).

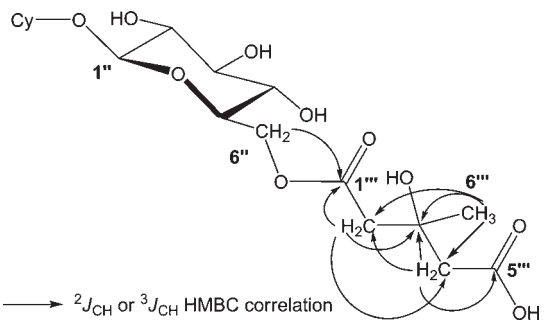


**Figure 4.** Highlighted NMR correlations observed in the $^1H-^{13}C$ HMBC spectrum of **5** used for identification of the acyl moiety as 3-hydroxy-3-methylglutaroyl.

**Table 3. Relative Amounts of Anthocyanins 1−5[a] in Wild Norwegian Blackberries and Three Blackberry Cultivars**

| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| wild Norwegian | 78.9 ± 0.2 | 4.8 ± 0.1 | 3.0[b] | 7.0 ± 0.1 | 6.3 ± 0.2 |
| Thornless Evergreen | 82.6 ± 0.1 | 3.1[b] | 5.7[b] | 4.5[b] | 4.1[b] |
| Lock Ness | 90.0 ± 0.1 | 5.5 ± 0.1 | 3.4[b] | 1.2[b] | tr[c] |
| Tupi | 87.7 ± 0.2 | 5.9 ± 0.2 | tr | 2.4 ± 0.1 | 3.9[b] |

[a] See Figure 2 for structures of **1**−**5**. [b] Standard deviations <0.1. [c] tr = trace amounts.

(**5**) in the genus of blackberries, we think, on the basis of HPLC profiles and spectroscopic data (high-resolution MS and NMR), that **5** most probably is the same pigment that has previously been reported to occur in various blackberry samples as cyanidin 3-dioxalylglucoside.

**Relative Amounts of Anthocyanins (1−5) in Wild Norwegian Blackberries and Three Blackberry Cultivars.** HPLC profiles showed that the anthocyanin content of the Norwegian wild blackberries and the cultivars Thornless Evergreen, Loch Ness, and Tupi were rather similar, with respect to both the kind of anthocyanins in the berries (**1**−**5**) and their relative amounts (Table 3). All five anthocyanins, **1**−**5**, were detected in all samples, and cyanidin 3-glucoside (**1**) was the major pigment, constituting 79−90% of the anthocyanin content. Cyanidin 3-(6″-(3-hydroxy-3-methylglutaroyl)glucoside) (**5**) constitutes 4−6% in the wild blackberries, Thornless Evergreen, and Tupi, whereas it was found only in trace amounts in Loch Ness.

## AUTHOR INFORMATION

### Corresponding Author

*Phone: +47-5558-3486. Fax: +47-5558-9490. E-mail: monica.jordheim@kj.uib.no.

## ACKNOWLEDGMENT

We are grateful to Sectional Engineer Terje Lygre, Department of Chemistry, University of Bergen, for recording of the LC-MS spectra.

## REFERENCES

(1) Andersen, Ø. M.; Jordheim, M. The anthocyanins. In *Flavonoids: Chemistry, Biochemistry and Applications*; Andersen, Ø. M., Markham, K. R., Eds.; CRC Press: Boca Raton, FL, 2006; pp 471−551.

(2) Andersen, Ø. M.; Jordheim, M. Chemistry of flavonoid-based colors in plants. In *Comprehensive Natural Products II: Chemistry and Biology*; Mander, L. N., Liu, H.-W., Eds.; Elsevier: Oxford, U.K., 2010; Vol. 3, pp 547−614.

(3) He, J.; Giusti, M. M. Anthocyanins: natural colorants with health-promoting properties. *Annu. Rev. Food Sci. Technol.* **2010**, *1*, 163–187.

(4) Rice-Evans, C.; Miller, N. Measurement of the antioxidant status of dietary constituents, low density lipoproteins and plasma. *Leukotrienes Essent. Fatty Acids* **1997**, *57*, 499–505.

(5) Moyer, R. A.; Hummer, K. E.; Finn, C. E.; Frei, B.; Wrolstad, R. E. Anthocyanins, phenolics, and antioxidant capacity in diverse small fruits *Vaccinium, Rubus,* and *Ribes*. *J. Agric. Food Chem.* **2002**, *50*, 519–525.

(6) Prior, R. L.; Hoang, H.; Gu, L.; Wu, X.; Bacchiocca, M.; Howard, L.; Hampsch-Woodill, M.; Huang, D.; Ou, B.; Jacob, R. Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity ($ORAC_{FL}$)) of plasma and other biological and food samples. *J. Agric. Food Chem.* **2003**, *51*, 3273–3279.

(7) Hassimotto, N. M. A.; Lajolo, F. M. Antioxidant status in rats after long-term intake of anthocyanins and ellagitannins from blackberries. *J. Sci. Food Agric.* **2011**, *91*, 523–531.

(8) Schantz, M.; Mohn, C.; Baum, M.; Richling, E. Antioxidative efficiency of an anthocyanin rich bilberry extract in the human colon tumor cell lines Caco-2 and HT-29. *J. Berry Res.* **2010**, *1*, 25–33.

(9) Mazza, G.; Miniati, E. *Anthocyanins in Fruits, Vegetables, and Grains*: CRC Press: Boca Raton, FL, 1993; pp 85−87.

(10) Sapers, G. M.; Hicks, K. B.; Burgher, A. M.; Hargrave, D. L.; Sondey, S. M.; Bilyk, A. Anthocyanin patterns in ripening thornless blackberries. *J. Am. Soc. Hortic. Sci.* **1986**, *111*, 945–950.

(11) Seeram, N. P.; Momin, R. A.; Nair, M. G.; Bourquin, L. D. Cyclooxygenase inhibitory and antioxidant cyanidin glycosides in cherries and berries. *Phytomedicine* **2001**, *8*, 362–369.

(12) Stintzing, F. C.; Stintzing, A. S.; Carle, R.; Wrolstad, R. E. A novel zwitterionic anthocyanin from Evergreen blackberry (*Rubus laciniatus* Willd.). *J. Agric. Food Chem.* **2002**, *50*, 396–399.

(13) Byamukama, R.; Kiremire, B. T.; Andersen, Ø. M.; Steigen, A. Anthocyanins from fruits of *Rubus pinnatus* and *Rubus rigidus*. *J. Food Comp. Anal.* **2005**, *18*, 599–605.

(14) Mertz, C.; Cheynier, V.; Guenata, Z.; Brat, P. Analysis of phenolic compounds in two blackberry species (*Rubus glaucus* and *Rubus adenotrichus*) by high-performance liquid chromatography with diode array eetection and electrospray ion trap mass spectrometry. *J. Agric. Food Chem.* **2007**, *55*, 8616–8624.

(15) Cuevas-Rodriguez, E. O.; Yousef, G. G.; Garcia-Saucedo, P. A.; Lopez-Medina, J.; Paredes-Lopez, O.; Lila, M. A. Characterization of anthocyanins and proanthocyanidins in wild and domesticated Mexican blackberries (*Rubus* spp.). *J. Agric. Food Chem.* **2010**, *58*, 7458–7464.

(16) Rommel, A.; Wrolstad, R. E.; Heatherbell, D. A. Blackberry juice and wine: processing and storage effects on anthocyanin composition, color and appearance. *J. Food Sci.* **1992**, *57*, 385–391.

(17) Siriwoharn, T.; Wrolstad, R. E.; Finn, C. E.; Pereira, C. B. Influence of cultivar, maturity, and sampling on blackberry (*Rubus* L. hybrids) anthocyanins, polyphenolics, and antioxidant properties. *J. Agric. Food Chem.* **2004**, *52*, 8021–8030.

(18) Fan-Chiang, H. J.; Wrolstad, R. E. Anthocyanin pigment composition of blackberries. *J. Food Sci.* **2005**, *70*, C198–C202.

(19) Talavera, S.; Felgines, C.; Texier, O.; Besson, C.; Gil-Izquierdo, A.; Lamaison, J. L.; Remesy, C. Anthocyanin metabolism in rats and their distribution to digestive area, kidney, and brain. *J. Agric. Food Chem.* **2005**, *53*, 3902–3908.

(20) Wu, X.; Prior, R. L. Systematic identification and characterization of anthocyanins by HPLC-ESI-MS/MS in common foods in the United States: fruits and berries. *J. Agric. Food Chem.* **2005**, *53*, 2589–2599.

(21) Ogawa, K.; Sakakibara, H.; Iwata, R.; Ishii, T.; Sato, T.; Goda, T.; Shimoi, K.; Kumazawa, S. Anthocyanin composition and cntioxidant activity of the crowberry (*Empetrum nigrum*) and other berries. *J. Agric. Food Chem.* **2008**, *56*, 4457–4462.

(22) Jordheim, M.; Måge, F.; Andersen, Ø. M. Anthocyanins in berries of *Ribes* including gooseberry cultivars with a high content of acylated pigments. *J. Agric. Food Chem.* **2007**, *55*, 5529–5535.

(23) Tatsuzawa, F.; Saito, N.; Mikanagi, Y.; Shinoda, K.; Toki, K.; Shigihara, A.; Honda, T. An unusual acylated malvidin 3-glucoside from flowers of *Impatiens textori* Miq. (Balsaminaceae). *Phytochemistry* **2009**, *70*, 672–674.