Quantification and Characterization of Anthocyanins in Balaton Tart Cherries

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The anthocyanin contents of Balaton and Montmorency cherries were compared. The results indicate that both cherries contain identical anthocyanins. However, Balaton contains approximately six times more anthocyanins than does Montmorency. Also, hydrolysis of the total anthocyanins and subsequent gas chromatographic (GC) and nuclear magnetic resonance (NMR) experiments with the resulting products indicated that both varieties contain only one aglycon cyanidin. This observation contrasts with existing reports of the presence of peonidin glycosides in Montmorency cherry. Results of the present study suggest that the anthocyanins in Balaton and Montmorency cherries are anthocyanin **1** [3-cyanidin 2″-*O*-*β*-D-glucopyranosyl-6″-*O*-α-L-rhamnopyranosyl-*β*-D-glucopyranoside], anthocyanin **2** [3-cyanidin 6″-*O*-α-L-rhamnopyranosyl-*β*-D-glucopyranoside], and anthocyanin **3** [3-cyanidin *O*-*β*-D-glucopyranoside].

Keywords: *Prunus cerasus; fruit; Balaton; Montmorency; anthocyanin; cyanidin; cyanidin glucoside; quantification and characterization*

INTRODUCTION

Prunus cerasus L. (Rosaceae) cv. Montmorency is the major tart cherry (commercially) grown in the United States. An artificial red dye is frequently added to Montmorency cherry food products to enhance its low natural red color. In order to diversify the Montmorency monoculture, a new Hungarian cultivar, Balaton tart cherry, was introduced into the United States in 1984, and has been tested in Michigan, Utah, and Wisconsin. Balaton produces fruits darker than Montmorency, and may be used as a source for cherry anthocyanins.

Natural pigments like anthocyanins were regarded as the index of quality in tart cherries (Mazza and Miniati, 1993). In addition, recent studies have demonstrated the strong antioxidant activities of anthocyanins such as cyanidin 3-glucoside (Tsuda et al., 1994). Antioxidants are commonly used to increase the shelf life of food products by preventing, or at least delaying, the onset of lipid peroxidation (Tsuda et al., 1994). Natural antioxidants may play an important role in the prevention of carcinogenesis. Dietary antioxidants may be effective against the preoxidative damage in living systems (Halliwell and Gutteridge, 1989; Osawa et al., 1990).

Early studies revealed that Montmorency cherry contains cyanidin 3-gentiobioside and cyanidin 3-rutinoside (Li and Wagenknecht, 1956). Cyanidin 3-glucosylrutinoside was also found in six out of seven tart cherry varieties analyzed by Harborne and Hall (1964). Cyanidin 3-glucoside is reported as a minor pigment in Montmorency cherries (Schaller and Von Elbe, 1968; Chandra et al., 1992). Dekazos (1970) reported anthocyanin pigments in Montmorency cherry as peonidin 3-rutinoside, peonidin and cyanidin along with cyanidin 3-sophoroside, cyanidin 3-rutinoside and cyanidin 3-glucoside (Schaller and Von Elbe, 1968). However, cyanidin 3-glucosylrutinoside as well as cyanidin 3-glucoside, cyanidin 3-sophoroside, and cyanidin 3-rutinoside were identified as the main pigments in tart cherries. Using high-performance liquid chromatography (HPLC) retention values, Chandra et al. (1992) reported that cyanidin 3-sophoroside and cyanidin 3-glucoside were the major and minor anthocyanins, respectively, in the Michigan-grown Montmorency cherries. Similarly, cyanidin 3-xylosylrutinoside was detected as a minor pigment in Montmorency cherry (Shrikhande and Francis, 1973). In addition to the comparison of anthocyanins in Balaton and Montmorency, this paper describes the isolation and characterization of anthocyanins in Balaton and Montmorency cherries by NMR, GC, and mass spectroscopic (MS) methods.

MATERIALS AND METHODS

Cherry Fruits. Pitted and frozen Montmorency and Balaton tart cherries were obtained from commercial growers (Traverse City, MI) through the Cherry Marketing Institute, Inc. (Okemos, MI). The cherries were flushed with nitrogen in freezer bags prior to their storage at −20 °C.

General Experimental. ^{1}H-NMR, ^{13}C-NMR and DQF COSY spectra were recorded on a Varian 500 and 300 MHz spectrometers using CD_3OD/DCl (μL) solution at 25 °C. All chemical shifts are given in parts per million relative to CD_3-OD (3.3 ppm). GC analyses were performed on an HP 5890 II (Hewlett Packard, Palo Alto, CA) using a DB-17 (30 m × 0.313 mm × 0.25 μm, J&W Scientific, Palo Alto, CA) column. The temperature program used was 150 °C, initial temperature held for 5 min, and then increased to 210 °C at 5 °C min^{-1}, maintained for 5 min, and finally to 270 °C at 5 °C min^{-1}. The injection port temperature was maintained at 250 °C. The flame ionization detector temperature was 300 °C and the carrier gas was helium at a linear flow velocity of 4 cm s^{-1} with a 1:70 split ratio. Fast atom bombardment-mass spectroscopy (FAB-MS) was carried out on a double focusing mass spectrometer in a glycerol matrix using Xe as reactant gas.

HPLC Conditions for Anthocyanin Analysis. All sample extracts (20 μL each) were analyzed on Chemcopak and

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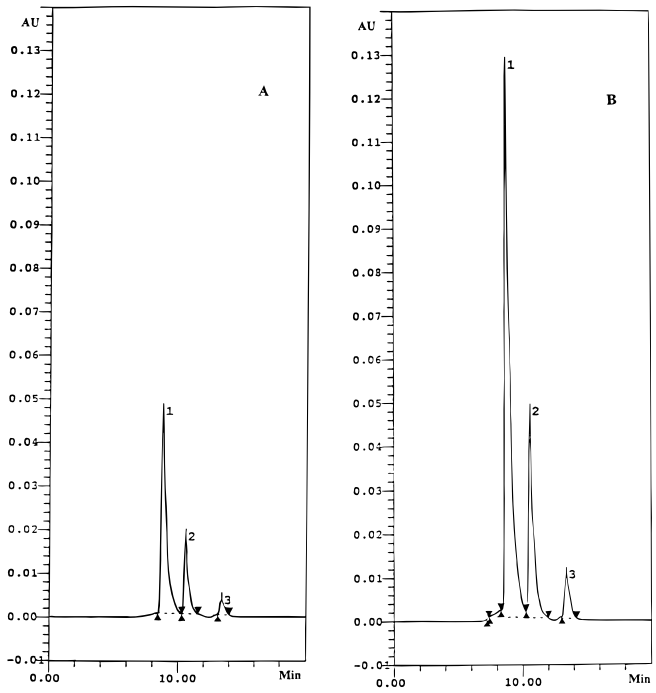

Figure 1. HPLC profile of Montmorency (A) and Balaton (B) cherry extracts: **1**, cyanidin 3-glucosylrutinoside; **2**, cyanidin 3-rutinoside; **3**, cyanidin 3-glucoside.

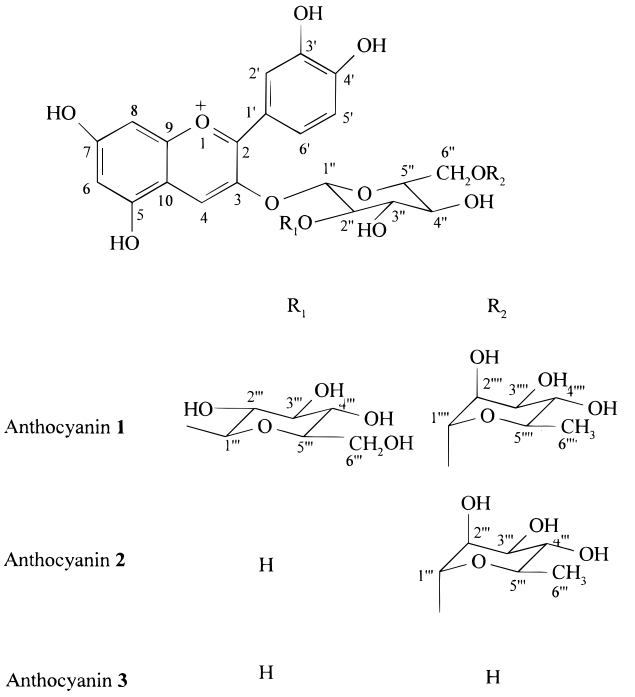

Figure 2. Anthocyanins **1**−**3** from Balaton cherries.

Capcellpak C-18 columns (10 × 250 mm, 5 μm) (Dychrom, Sunnyvale, CA). The mobile phase (4% aqueous H_3PO_4/CH_3-CN (80:20 v/v) was used under isocratic conditions at a flow rate of 1.5 mL min^{-1}. The anthocyanins were detected at 520 nm using a Waters PDA detector (Waters Corp., Milford, MA). Anthocyanins **1**−**3**, 0.5 mg each, were weighed and dissolved in 1 mL of H_2O/CH_3CN (1:1). The solutions were prepared by the serial dilution of the respective stock solutions to afford 0.25, 0.20, 0.10, 0.05, 0.025, and 0.0125 mg/mL concentrations, respectively. Quantification of anthocyanins was carried out using a Millennium 2010 chromatography manager (Waters Corp.).

Isolation of Crude Anthocyanins from Tart Cherries. The pitted cherries (400 g each of Balaton and Montmorency) were homogenized separately for 10 min using a Kinematica CH-6010 (Roxdale, Ontario, Canada) homogenizer and centrifuged (Model RC5C, Sorvall Instruments, Hoffman Estates, IL) at 10000*g* for 10 min at 4 °C to separate insoluble materials from the supernatant. The supernatant (400 mL each) was applied to an XAD-2 column (100 g, amberlite resin, mesh size 20−50; Sigma Chemical Co., St. Louis, MO), which was prepared as described by Chandra et al. (1993). The column was washed with H_2O (9 L) until the colorless washings gave a pH of about 7. The adsorbed pigments were then eluted with methanol (500 mL). The red methanolic solution was concentrated at 50 °C *in vacuo*, and the aqueous solution was lyophilized to yield an amorphous red anthocyanin powder, 0.86 and 0.54 g, respectively, for Balaton and Montmorency samples.

HPLC Analysis of Anthocyanins in Cherries. Pitted cherries (100 g) were homogenized and centrifuged as described above. The supernatant was decanted and adjusted with H_2O to a final volume of 250 mL in a volumetric flask. An aliquot of 1 mL of this solution was passed through a preconditioned C-18 Sep-Pak cartridge (Waters Associates). The adsorbed pigments were then washed with 2 mL of water followed by 1 mL of H_2O/CH_3CN (1:1). The eluate was stored at −20 °C prior to HPLC analysis. Both Balaton and Montmorency showed identical HPLC profiles (Figure 1).

Purification of Anthocyanins 1−3. The crude anthocyanins (Figure 2) from Balaton were fractionated by C-18 medium-pressure liquid chromatography (MPLC) to produce pure anthocyanins. The anthocyanin mixture (350 mg) was dissolved in water (2 mL), injected into the C-18 column (40 × 500 mm), and eluted with 4% H_3PO_4/CH_3CN (80:20). Four fractions, I: 125 mL, II: 100 mL, III: 100 mL, and IV: 275 mL, were collected and evaporated under reduced pressure. The H_3PO_4 from these fractions was removed by passing each fraction through preconditioned C-18 Sep-Pak cartridges with methanol, followed by 10% methanol. The adsorbed pigment was washed with 5 mL water to remove the acids and then eluted with 5 mL of H_2O/methanol (1:1) to afford pure anthocyanins. The yields of anthocyanins from fractions I−IV were 53, 24, 133, and 64 mg, respectively. HPLC analysis of these fractions revealed that fraction I was pure and contained only anthocyanin **1**. Fraction II contained anthocyanins **1** and **2**, fraction III had anthocyanins **2** and **3**, and fraction IV contained anthocyanin **3** with other phenolics as indicated by their HPLC profiles.

Since fractions II and III from MPLC contained all three of the anthocyanins, 40 mg of II and 30 mg of III were purified further by HPLC on Capcellpak C-18 column (10 × 250 mm, 5 μm) to yield pure anthocyanins **2** and **3**. Peaks were detected using a PDA detector at 520 and 283 nm, respectively. The mobile phase (4% aqueous H_3PO_4/CH_3CN, 83:17 v/v) was used under isocratic conditions at a flow rate of 2.0 mL/min. Respective anthocyanin fractions from HPLC purification of fractions II and III were combined, dried under reduced pressure, and purified further using C-18 Sep-Pak to remove H_3PO_4. The weights of pure anthocyanins **1**−**3** were 5.7, 8.9, and 2.9 mg, respectively.

Crude anthocyanins from Montmorency (500 mg) were also fractionated by C-18 MPLC as in the case of Balaton. Three bands with red color were collected as fractions I, II, and III and removal of solvents at reduced pressure afforded 10, 30, and 20 mg of anthocyanins, respectively. Fraction I was pure and contained only anthocyanin **1**. Fractions II and III were not pure by HPLC analysis and contained anthocyanins **1**−**3**.

The purified anthocyanins are red amorphous powders (Figure 2). Complete assignments of ^{1}H- and ^{13}C-NMR spectra of pure anthocyanins **1** [3-cyanidin 2″-*O*-β-D-glucopyranosyl-6″-*O*-α-L-rhamnosyl-β-D-glucopyranoside], **2** [3-cyanidin 6″-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside], and the ^{1}H-NMR of **3** [3-cyanidin β-D-glucopyranoside] are given in Tables 1 and 2, respectively. ^{13}C-NMR on pure anthocyanin **3** was not performed due to its low yield.

Cyanidin, the Aglycon. The crude anthocyanin powder from Balaton (55 mg) was hydrolyzed with 3 M HCl (15 mL) for 1 h at 100 °C. The red solution was cooled to room temperature and stirred with 1-butanol (20 mL). The mixture

Table 1. ^{1}H-NMR Chemical Shifts in Parts per Million for Anthocyanins 1−3 and Their Aglycon in CD_3OD/DCl (*J* in Hertz)

proton	1	2	3	aglycon
H-4	8.89 s	8.92 s	8.98 s	8.62 s
H-6	6.67 d (1.96)	6.69 d (1.95)	6.71 d (1.95)	6.65 d (1.95)
H-8	6.90 d (1.96)	6.91 d (1.95)	6.98 d (1.95)	6.90 d (1.95)
H-2′	8.00 d (2.24)	8.02 d (2.23)	8.05 d (2.23)	8.11 d (2.23)
H-5′	7.06 d (8.66)	7.01 d (8.65)	7.07 d (8.65)	7.02 d (8.66)
H-6′	8.18 dd (8.66, 2.24)	8.27 dd (8.65, 2.23)	8.29 d (8.65, 2.23)	8.17 dd (8.66, 2.23)
H-1″	5.43 d (7.29)	5.29 d (7.53)	5.40 d (7.50)	
H-2″	4.05 dd (9.08, 7.29)	3.67 dd (9.06, 7.53)	3.67 dd (9.00,7.50)	
H-3″	3.77 dd (9.28, 9.08)	3.55 dd (9.22, 9.06)	3.55 dd (9.22, 9.00)	
H-4″	3.50 dd (9.53, 9.28)	3.34 dd (9.49, 9.22)	3.34 dd (9.50, 9.22)	
H-5″	3.72 ddd (9.53, 6.41, 1.76)	3.71 m	3.71 m	
H-6″a	4.04 dd (12.23, 6.41)	4.05 dd (11.90, 6.31)	3.91 dd (11.90, 6.30)	
H-6″b	3.61 dd (12.23, 1.76)	3.62 dd (11.90, 1.62)	3.68 dd (11.90, 1.62)	
H-1‴	4.76 d (7.74)	4.65 d (1.67)		
H-2‴	3.19 dd (9.08, 7.74)	3.80 dd (3.35, 1.67)		
H-3‴	3.33 dd (9.08, 9.28)	3.63 dd (9.49, 3.35)		
H-4‴	3.23 t (9.28)	3.41 dd (9.49, 9.21)		
H-5‴	2.92 dt (9.28, 3.97)	3.54 dd (9.21, 6.14)		
H-6‴	3.44 d (3.97)	1.15 d (6.14)		
H-1⁗	4.65 d (1.54)			
H-2⁗	3.78 dd (3.31, 1.54)			
H-3⁗	3.60 dd (9.50, 3.31)			
H-4⁗	3.27 dd (9.28, 9.50)			
H-5⁗	3.56 dd (9.28, 6.18)			
H-6⁗	1.14 d (6.18)			

Table 2. ^{13}C-NMR Chemical Shifts in Parts per Million for Anthocynains 1 and 2 and Their Aglycon in CD_3OD/DCl

carbon	1	2	aglycon
C-2	164.3	164.3	162.6
C-3	145.2	145.6	146.5
C-4	136.1	136.6	134.0
C-5	159.0	159.0	157.9
C-6	103.5	103.5	103.0
C-7	170.4	170.4	168.8
C-8	95.2	95.3	94.7
C-9	157.6	157.6	156.9
C-10	113.2	113.2	113.5
C-1′	121.2	121.2	121.9
C-2′	118.6	118.4	117.9
C-3′	147.4	147.4	147.3
C-4′	155.7	155.8	155.1
C-5′	117.6	117.5	117.2
C-6′	128.3	128.4	127.1
C-1″	104.9	103.5	
C-2″	82.3	74.7	
C-3″	77.2	77.4	
C-4″	71.2	71.2	
C-5″	77.9	78.0	
C-6″	67.6	67.8	
C-1‴	101.9	102.1	
C-2‴	75.9	71.9	
C-3‴	77.7	72.4	
C-4‴	70.8	73.9	
C-5‴	77.7	69.7	
C-6‴	62.3	17.9	
C-1⁗	102.2		
C-2⁗	71.8		
C-3⁗	72.4		
C-4⁗	73.9		
C-5⁗	69.8		
C-6⁗	17.9		

was extracted with water (3 × 20 mL). The combined water extracts were evaporated to dryness at reduced pressure to yield the sugars (30 mg). The red butanol layer was evaporated to dryness (24.3 mg). The residue was purified by silica gel preparative TLC using the solvent system, ethyl acetate/formic acid/2 M HCl , 85:6:9. The single red band at R_f 0.28 was eluted with MeOH, evaporated under reduced pressure, and afforded a red amorphous powder, cyanidin (11.2 mg). Similarly, pure anthocyanins (0.5 mg each) were hydrolyzed to obtain their respective sugars for GC analysis. The aglycons from anthocyanins **1**−**3** and the crude anthocaynin gave identical R_f values and HPLC retention times. Also, all aglycons showed identical ^{1}H- and ^{13}C-NMR spectra (Tables 1 and 2).

Characterization of Sugars by GC Analysis. The sugar standards rhamnose, fructose, galactose, glucose, and the internal standard phenyl-β-D-glucoside (E. Merck, Darmstadt, Germany) (1 mg each) and the sugars obtained from the hydrolysis of crude and pure anthocyanins (**1**−**3**) (1 mg each) were reacted separately with 30 mg/mL hydroxylamine HCl in dry pyridine (2 mL). The resulting oximes were then reacted with 1.0 mL hexamethyldisilazane (HMDS) and 0.1 mL trifluoroacetic acid (TFA) to yield their silyl derivatives. The samples were then analyzed by GC using an autosampler. Sugars from anthocyanins **1**, **2**, and **3** were identified by comparison with the retention times of sugar standards. The retention times were 6.97, 9.83, 10.58, 10.90, and 19.82 min, respectively, for rhamnose, fructose, galactose, glucose, and phenyl-β-D-glucoside. The GC analysis of sugars yielded from the hydrolysis of anthocyanins showed that anthocyanin **1** contained a 2:1 ratio of glucose and rhamnose. Anthocyanin **2** showed a 1:1 ratio of glucose and rhamnose, and anthocyanin **3** afforded only glucose. Similarly, the GC analysis of sugars from the crude anthocyanin powder indicated that it contained only rhamnose and glucose at a ratio of 2:4, respectively.

RESULTS AND DISCUSSION

Lyophilization of 100 g each of Balaton and Montmorency cherries afforded 17.1 and 14.7% of dry weights, respectively. The concentrations of the sugars and acids in Balaton were about 50% more than that in Montmorency cherries (data not presented). Similarly, total anthocyanin concentration in Balaton cherry is about six times higher than that in Montmorency cherry based on anthocyanin concentrations in fractions obtained from MPLC and HPLC purifications.

Prior to the isolation of anthocyanins for spectral characterization, both Balaton and Montmorency cherries were analyzed by HPLC under identical conditions. HPLC profiles of the cherry extract showed that there are two major and one minor anthocyanins in both varieties as indicated by retention times 8.76, 10.58, and 13.38 min, respectively, for anthocyanins **1**−**3** (Figure 1). Also, it was evident from the marked difference in the red color between these two cherries and from HPLC

profiles (Figure 1) that Montmorency contained relatively smaller amounts of anthocyanins compared to Balaton.

Production of pure anthocyanins (**1**−**3**) from Balaton and Montmorency cherry juices was carried out first by adsorbing the pigment on an Amberlite XAD-2 column (Chandra et al., 1993). The column was washed with water until the eluant gave a pH of approximately 7.0. The adsorbed pigments along with other phenolics were eluted with MeOH. The resulting crude anthocyanins were fractionated and purified by C-18 MPLC and HPLC, respectively, to afford pure anthocyanins for spectral studies. Purification of 500 mg crude Montmorency anthocyanins from XAD-2 yielded 60 mg of anthocyanins **1**−**3** (Figure 2) compared to 391.43 mg from Balaton. This indicates that crude anthocyanins from Montmorency obtained from the XAD-2 contained a higher percentage of other organic compounds.

The presence of cyanidin and respective sugar moieties in anthocyanins **1**−**3** was confirmed by the comparison of their ^{1}H- and ^{13}C-NMR chemical shifts with published data (Glässgen et al., 1992; Strack and Wray, 1989). We have determined the relative configuration and nature of the sugars in anthocyanins **1** and **2** by DQF COSY and from the vicinal and geminal ^{1}H−^{1}H coupling constants.

The ^{1}H-NMR spectrum of **1** (Table 1) gave signals for three anomeric protons that appeared at δ 5.43, 4.76, and 4.65, respectively, for glucose (attached to the aglycon), glucose, and rhamnose. Also, the presence of β-D-glucosidic linkage in **1** was confirmed by the large coupling constants for the anomeric protons (Table 1). The signal at δ 4.65 ppm corresponded to the anomeric proton of an L-rhamnopyranose and the 1.8 Hz coupling constant indicated an α-glycosidic linkage.

The ^{13}C-NMR chemical shifts observed for anthocyanins in Balaton and Montmorency were similar to the published data (Agrawal et al., 1989). The C-7 resonated at very low field 170.4 ppm compared to the rest of the oxygenated aromatic carbons in anthocyanins. The oxygen cation in ring C is responsible for the downfield shift of C-7 carbon. The ^{13}C-NMR signal for C-5 carbon in **1** at δ 69.8 confirmed the rhamnosyl moiety with an α-linkage to the glucose (Agrawal, 1992).

The downfield shift of the C-2″ proton in **1** relative to the C-2″ signal of **2** (Table 2) was due to the glucosylation and indicated a 1,2 linkage between the two glucose units. Similarly, the downfield shift of C-6″ proton in the ^{1}H-NMR spectrum of **1** (Table 1) relative to the C-6 proton signal of glucose was due to the rhamnose moiety and indicated a 1,6 linkage between the glucose and rhamnose. Anthocyanin **1** gave a molecular ion at *m*/*z* 758 $[M + H]^+$ and the base peak at *m*/*z* 596 $[M + H\text{-}C_6H_{10}O_5]$ in the FAB−MS also indicated the presence of cyanidin, and two glucose and one rhamnose moieties in **1**. Therefore, anthocyanin **1** is confirmed to be 3-cyanidin 2″-*O*-β-D-glucopyranosyl-6″-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside.

^{1}H-NMR spectrum of **2** (Table 1) showed signals for two anomeric protons at δ 5.29 (J = 7.53 Hz) and 4.65 (J = 1.67 Hz). This indicated the presence of a β-D-glucose because all vicinal coupling constants were 7.53−11.9 ppm. The doublet (J = 6.14 Hz) at 1.15 ppm of a methyl group confirmed one of the sugars as rhamnose in **2**. The small coupling constant of 1.67 Hz for the anomeric proton suggested an α-rhamnosyl linkage. The C-6″ carbon in **2** appeared at 67.8 ppm indicated a 1,6 linkage between the glucose and rhamnose. The FAB-MS of **2** gave the molecular ion at *m*/*z* 596 $[M + H]^+$ and confirmed its structure as 3-cyanidin 6″-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside.

The ^{1}H-NMR of anthocyanin **3** (Table 1) revealed only a single glucose moiety attached to the aglycon cyanidin. The structure of **3** was confirmed to be 3-cyanidin β-D-glucoside. Hydrolysis of crude anthocyanins and TLC of resulting products as well as ^{13}C-NMR data showed that the only aglycon present in both Balaton and Montmorency cherries was cyanidin.

Our results suggest that there are only three identical anthocyanins present in both Balaton and Montmorency cherries. The yields of spectroscopically pure anthocyanins **1**−**3** in 100 g of fresh Balaton and Montmorency cherries were 14.99, 6.20 ; 6.18, 0.97; and 2.42, 0.35 mg, respectively. The amount of anthocyanins isolated from Montmorency in our studies show that it is lower than the reported yields (Dekazos, 1970). However, this may be due to varying environmental and nutritional factors. An important point to note is that when anthocyanins are monitored by HPLC at 520 nm, other phenolic compounds which absorb at 283 nm are ignored. We have isolated at least four phenolic compounds co-eluted with the anthocyanins and detected at 283 nm.

Chandra et al. (1992) reported that Montmorency cherries grown in Michigan contain only cyanidin 3-sophoroside and cyanidin 3-glucoside. These results were confirmed by matching their retention times to those of the anthocyanins present in an authentic sample of blackberry juice described by Hong and Wrolstad (1990a,b). Also, earlier reports indicated that there are peonidin 3-glycoside and peonidin 3-galactoside present in Montmorency cherry (Chandra et al., 1992). However, our present study indicates that Montmorency contains the same number of anthocyanins found in Balaton cherries and that the anthocyanins were identical. This is the first report of the characterization of anthocyanins from Balaton cherries and the spectral characterization of anthocyanins from Montmorency cherries.

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LITERATURE CITED

Agrawal, P. K.; Mahesh C. B. Flavonoid Glycoside. In *Carbon-13 NMR of Flavonoids*, Agrawal, P. K., Ed.; Elsevier: Amsterdam, 1989, 283−364.

Agrawal, P. K. NMR spectroscopy in the structural elucidation of oligosaccharides and glycoside. *Phytochemistry* **1992**, *31*, 3307−3330.

Chandra,A.; Nair, M. G.; Iezzoni, A. Evaluation and characterization of the anthocyanin pigment in tart cherries (*Prunus cerasus* L.). *J. Agric. Food Chem.* **1992**, *40*, 967−969.

Chandra, A.; Nair, M. G.; Iezzoni, A. Isolation and stabilization of anthocyanins from tart cherries (*Prunus cerasus* L.). *J. Agric. Food Chem.* **1993**, *41*, 1062−1065.

Dekazos, E. D. Anthocyanin pigments in red tart cherries. *J. Food Sci.* **1970**, *35*, 237−241.

Glässgen, W. E.; Wray, V.; Strack, D.; Metzger, J. W.; Seitz, H. U. Anthocyanins from cell suspension cultures of *Dacus carota*. *Phytochemistry* **1992**, *31*, 1593−1601.

Halliwell, B.; Gutteridge, J. M .C. *Free Radicals in Biology and Medicine*; Oxford University Press: New York, 1989; pp 416−494.

Harborne, J. B.; Hall, E. Plant polyphenols. XIII. The systematic distribution and origin of anthocyanins containing branched trisaccharides. *Phytochemistry* **1964**, *3*, 453−463.

Hong, V.; Wrolstad, R. E. Characterization of anthocyanin-containing colorants and fruit juices by HPLC/photodiode array detection. *J. Agric. Food Chem.* **1990a**, *38*, 698−708.

Hong, V.; Wrolstad, R. E. Use of HPLC separation/photodiode array detection for characterization of anthocyanins. *J. Agric. Food Chem.* **1990b**, *38*, 708−715.

Li, K. C.; Wagenknecht, A. C. The anthocyanin pigments of sour cherries. *J. Am. Chem. Soc.* **1956**, *78*, 979−980.

Mazza, G.; Miniati, E. *Anthocyanins in Fruits, Vegetables, and Grains*; CRC Press: Boca Raton, Florida, 1993; 57−63.

Osawa, T.; Namiki, M.; Kawakishi, S. Role of dietary antioxidants in protection against oxidative damage. In *Antimutagenesis and Anticarcinogenesis Mechanisms*; Kuroda, Y., Shankel, D. M., Waters, M. D., Eds.; Plenum Publishing: New York, 1990; 139−153.

Schaller, D. R.; Von Elbe, J. H. The minor pigment component of Montmorency cherries. *J. Food Sci.* **1968**, *3*, 442−443.

Shrikhande, A. J.; Francis, F. J. Anthocyanin pigments of sour cherries. *J. Food Sci.* **1973**, *38*, 649−651.

Strack, D.; Wray, V. In *Methods in Plant Biochemistry*, vol. 1; Phenolics; Anthocyanins; Harborne, J. B. Ed.; Academic Press: London, 1989; 325−356.

Tsuda, T.; Watanabe, W.; Ohshima, K.; Norinobu, S.; Choi, S.; Kawakishi, S.; Osawa, T. Antioxidative activity of the anthocyanin pigments Cyanidin 3-O-β-D-glucoside and cyanidin. *J. Agric. Food Chem.* **1994**, *42*, 2407−2410.

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