Evaluation and Characterization of the Anthocyanin Pigments in Tart Cherries (*Prunus cerasus* L.)

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A novel method has been developed for the extraction, isolation, and separation of the anthocyanins in tart cherry (*Prunus cerasus* L.) cultivars. The presence of cyanidin 3-sophoroside, cyanidin 3-glucosylrutinoside, cyanidin 3-glucoside, and cyanidin 3-rutinoside was confirmed in Montmorency, English Morello, and three Michigan State University hybrids, II 7 (30), I 21 (33), and II 9 (11). Also, peonidin 3-galactoside is reported for the first time to be one of the color pigments present in tart cherries. A higher level of anthocyanins is shown in all of the hybrid selections studied in contrast to the levels in Montmorency and English Morello. The HPLC profiles of anthocyanin as described in this paper can serve as a qualitative reference for the different anthocyanins in cherry juices and concentrates.

INTRODUCTION

Prunus cerasus L. (Rosaceae), cv. Montmorency, is the most important tart cherry cultivar in the United States. An artificial red dye is frequently added to Montmorency cherry food products to enhance its low natural red color. There is interest in identifying tart cherry cultivars with high levels of anthocyanin pigments, such as the European cultivar, English Morello, to blend with Montmorency fruits to enhance the color. Some of the selections from the Michigan State University (MSU) tart cherry breeding program produce cherries darker than Montmorency and English Morello. Because of the importance of the red pigments, cherry anthocyanins have been investigated by researchers worldwide (Dekazos, 1970; Shrikhande and Francis, 1973; Francis, 1989; Hong and Wrolstad, 1990a) and may therefore provide a more efficient cherry colorant.

Anthocyanins, one of the major group of pigments, are responsible for the orange, red, and blue colors in fruits, vegetables, flowers, and other storage tissues in plants. Their presence is universally associated with attractive, colorful, and flavorful fruits. Natural anthocyanins can be considered as alternatives to synthetic food colorants and therefore are of special interest to the food industry. Because of the uniqueness of anthocyanin profiles, they can be used to identify specific fruits and fruit products to determine product authenticity (Wrolstad et al., 1981).

The visual appeal of tart cherries (*P. cerasus* L.) is due to their brilliant red color. The anthocyanins in the fruit of the European cherry cultivar, English Morello, have been characterized (Hong and Wrolstad, 1990a). The tart cherry cultivar Montmorency, the only tart cherry commercially grown in the United States, has been studied for its anthocyanin contents (Von Elbe and Schaller, 1968; Shrikhande and Francis, 1973). Unlike English Morello, which has red juice, Montmorency has clear juice. Our preliminary observations indicate that several selections in the MSU tart cherry breeding program have pigment levels higher than that of English Morello; these may be of interest as a source of pigments to enhance the color of Montmorency fruit products. Our objectives were to develop a comparatively simple method for the extraction and analysis of the anthocyanin pigments from various cherry samples and characterize them.

MATERIALS AND METHODS

Cherry Samples. Montmorency and English Morello cherries were obtained from commerical growers in Michigan. The MSU tart cherry hybrid selections II 7 (30), II 9 (11), and I 21 (33) were grown and fruits collected from the Clarksville Horticultural Experimental Station (Clarksville, MI). The cherries were flushed with nitrogen in freezer bags, prior to their storage at −20 °C.

Juice Concentrates. Cranberry and blackberry juice concentrates were obtained from Milne fruit products (Prosser, WA) and Kerr Concentrates (Salem, OR), respectively.

Extraction of the Pigments. Pitted cherries (10 g) were homogenized separately in water (10 mL) for 5 min in a Kinematica CH-6010 (Kriens-LU) homogenizer and centrifuged individually (RC5C centrifuge, Sorvall Instruments) at 10000*g* for 10 min at 4 °C. The supernatant juices were stored at −20 °C as stock solutions for the analyses.

Sampling for the HPLC Analysis. The juice concentrates of blackberry and cranberry, 1 mL each, were further diluted with water (6×), to obtain a stock solution of equivalent single-stength juice samples. The stock solution (600 μL) from the cherry samples and the samples from blackberry and cranberry were adsorbed on a preconditioned (1:1 H_2O/CH_3CN) C-18 Sep-Pak (Waters Associates). The adsorbed pigments were then subsequently washed with 0.10% aqueous H_3PO_4 solution (2 mL) followed by water (2 mL) and then were eluted with H_2O/CH_3CN (1:1, 1 mL) and stored at −20 °C prior to HPLC analyses.

HPLC Conditions. All samples (5 μL each) were analyzed on Chemcopak and Capcellpak (Dychrom; Sunnyvale, CA) C-18 columns (10 × 250 mm, 5 μm). The mobile phase (4% aqueous H_3PO_4/CH_3CN (80:20 v/v) was used under isocratic conditions at a flow rate of 1.5 mL/min. The samples were analyzed at 520 nm using a Waters 490 variable-wavelength UV–visible detector (Waters).

Identification of Anthocyanins. Anthocyanins A1–4 were identified by matching their retention times to those of the anthocyanins present in an authentic sample of blackberry juice, as described by Hong and Wrolstad (1990a,b). Anthocyanin A5 (peonidin 3-galactoside) is detected by matching the test samples with an authentic sample of cranberry juice concentrate (Hong and Wrolstad, 1986, 1990b). The anthocyanins are expressed as peak area percentages from the respective HPLC analysis (Figures 1–5).

RESULTS AND DISCUSSION

Anthocyanins exist in aqueous solution as equilibrium mixtures of four structural forms: the flavylium cation AH^+ (red), the pseudobase or carbinol form (colorless),

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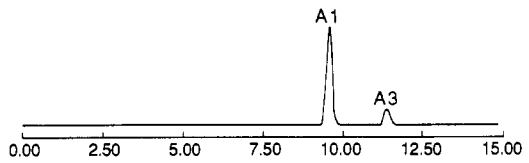


Figure 1. HPLC chromatogram of Montmorency cherry anthocyanins. Peak area percentages are as follows: A1, 86.64%; A3, 13.36%.

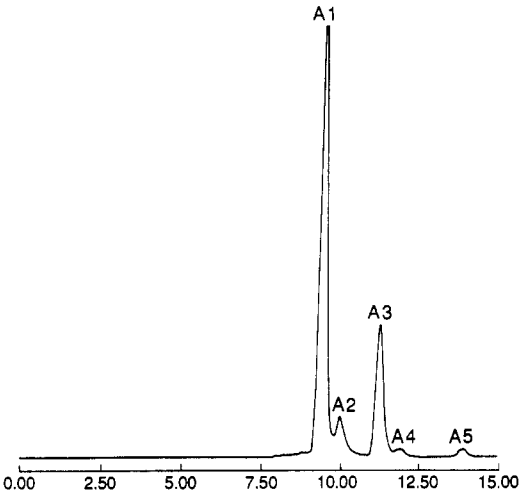


Figure 2. HPLC chromatogram of English Morello cherry anthocyanins. Peak area percentages are as follows: A1, 67.89%; A2, 8.64%; A3, 23.47%; A4, A5, less than 1%.

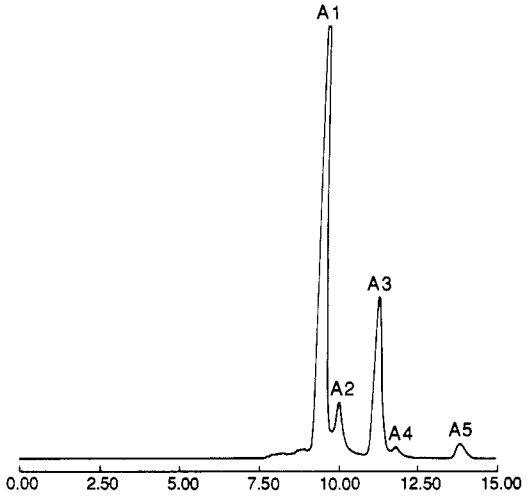


Figure 3. HPLC chromatogram of II 7 (30) cherry anthocyanins. Peak area percentages are as follows: A1, 59.91%; A2, 10.95%; A3, 24.24%; A4, 1.99%; A5, 2.91%.

the chalcone (pale yellow), and the quinonoidal (pale purple). The chalcone form is reported to be most susceptible to degradation (Brouillard et al., 1982). The formation of the flavylium cation and its predominance in the equilibrium mixture is considered to be the main reason for the bright red color in cherries (Francis, 1989). The existence of anthocyanin as equilibrium mixtures in four different structural forms complicates the analysis and identification of these pigments. Therefore, a low pH is essential to keep the anthocyanins in AH^+ form which is red in color and comparatively more stable than the other forms (Skrede, 1985; Van Buren et al., 1960). Also, the red color of anthocyanin (AH^+) is known to enhance peak sharpness, thereby making UV–visible detection of these pigments more feasible (Hale et al., 1986).

In most cases anthocyanins are analyzed by HPLC under

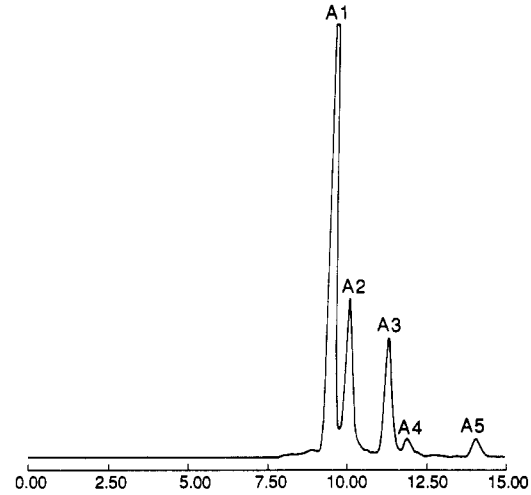


Figure 4. HPLC chromatogram of I 21 (33) cherry anthocyanins. Peak area percentages are as follows: A1, 56.13%; A2, 22.06%; A3, 16.03%; A4, 2.95%; A5, 2.83%.

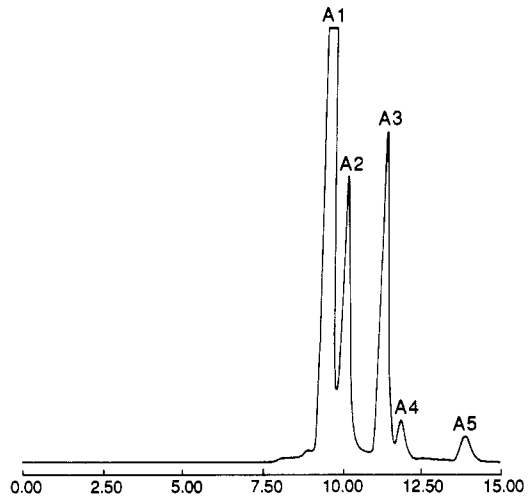


Figure 5. HPLC chromatogram of II 9 (11) cherry anthocyanins. Peak area percentages are as follows: A1, 57.24%; A2, 18.44%; A3, 19.31%; A4, 2.90%; A5, 2.11%.

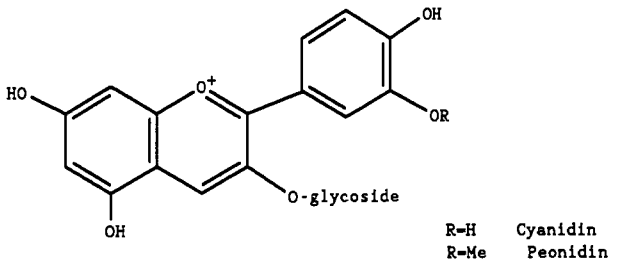
gradient conditions (Hong and Wrolstad, 1990a,b). The use of HCl in the mobile phase was avoided during the isolation of the pigments from cherries because it may result in artifacts and varying proportions of anthocyanins during the analyses (Von Elbe and Schaller, 1968). We used aqueous H_3PO_4 (4%) and CH_3CN (80:20) as the mobile phase under isocratic conditions to achieve a faster separation, thereby reducing the risk of possible degradation of these pigments under analytical conditions. The pigments were purified by adsorbing them on a C-18 Sep-Pak prior to the HPLC analyses. This methodology has improved the efficiency and reliability of the extraction of anthocyanins from intact cherries and made the analysis much faster and convenient.

The HPLC analysis showed two major peaks for Montmorency at RT 9.52 and 11.38 min, respectively (Figure 1; Table I). English Morello had three major peaks, and the MSU selections II 7 (30), I 21 (33), and II 9 (11) gave five peaks each (Figures 2–5; Table I). English Morello and II 9 (11) had 6 and 19 times the total pigment content, respectively, compared to Montmorency (Table I). We report cyanidin 3-sophoroside (A1), cyanidin 3-glucosylrutinoside (A2), cyanidin 3-glucoside (A3), and cyanidin 3-rutinoside (A4) as the anthocyanins present in our tart cherry samples, in agreement with the results published by Hong and Wrolstad (1990a). Our analysis also indicated

Table I. Level of Anthocyanins A1–5[a] Obtained from HPLC Analysis in Five Tart Cherry Selections

selection	A1	A2	A3	A4	A5	total
Montmorency	1.88		0.29			2.17
English Morello	7.78	0.99	2.69	Tr	Tr	11.46
II 7 (30)	8.43	1.54	3.41	0.28	0.41	14.07
I 21 (33)	8.93	3.51	2.55	0.47	0.45	15.91
II 9 (11)	20.33	6.55	6.86	1.03	0.75	35.52

[a] A1, cyanidin 3-sophoroside; A2, cyanidin 3-glucosyl rutinoside; A3, cyanidin 3-glucoside; A4, cyanidin 3-rutinoside; A5, peonidin 3-glactoside. [b] Tr, peak area <0.20.



A1 Cyanidin 3-sophoroside
A2 Cyanidin 3-glucosyl rutinoside
A3 Cyanidin 3-glucoside
A4 Cyanidin 3-rutinoside
A5 Peonidin 3-galactoside

Figure 6. Tart cherry anthocyanins.

the presence of peonidin 3-galactoside (A5) in the cherry samples analyzed (Figure 6).

Recently, Hong and Wrolstad (1990a) reported cyanidin 3-glucosylrutinoside as the major anthocyanin present in English Morello. Cyanidin 3-sophoroside, cyanidin 3-glucosylrutinoside, cyanidin 3-glucoside, and cyanidin 3-rutinoside along with peonidin 3-glucoside and peonidin 3-rutinoside were the anthocyanins reported to be present in various cultivars of cherries (Harborne and Hall, 1964; Dekazos, 1970; Shrikhande and Francis, 1973). We found A1 (cyanidin 3-sophoroside) as the major component in all of the cherry samples analyzed (Table I).

This is also the first report of the HPLC profiles of anthocyanins from various commercial cultivars and hybrid varieties of tart cherries. The results indicate that all tart cherry samples contain sophorose, glucosylrutinose, glucose, rutinose, and galactose as the sugar moieties associated with cyanidin and peonidin. The sample preparation and the HPLC analysis of the anthocyanins described in this paper can be used as a reference to characterize the anthocyanins of other related plant-derived pigments. The red pigments in tart cherries could be developed as a natural red dye for its application in the food industry. A thorough understanding of the anthocyanin pigments in these hybrids may lead to the selection of the tart cherry cultivar or the hybrids with high pigment content for future plantings and may be very important to the tart cherry industry in Michigan and elsewhere. As evident from these results, Montmorency had a low pigment concentration compared to English Morello and the MSU hybrid selections (Table I). However, English Morello, a commerical variety in Europe, shows lower anthocyanin content when compared to that of the MSU selections II 7 (30), I 21 (33), and II 9 (11). The juices from MSU tart cherry selections containing high anthocyanin concentration could be blended with Montmorency or English Morello to enhance their juice colors.

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