Proportions of Individual Anthocyanins in Fruits of Cranberry Cultivars

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Abstract. **Variation in the proportions of individual anthocyanins in cranberry (*Vaccinium macrocarpon* Ait.) fruit of different coloration was investigated. Light-, medium-, and dark-red subsamples of 'Franklin', 'McFarlin', and 'Searles' cranberries were analyzed to determine the soluble solids : acidity ratio (SS:A), total anthocyanin, and individual anthocyanins. Qualitative and quantitative differences in the pattern of individual anthocyanins among clones studied over three seasons were not significant. Proportions of individual anthocyanins in fruit subsamples of different coloration within cultivar samples also were similar, indicating constant biosynthetic rates for each anthocyanin during color development. Linear relationships between HPLC peak areas for individual anthocyanins and subsample total anthocyanin or SS:A values were observed.**

The red color of cranberry fruit is due to the presence of four major anthocyanin pigments: cyanidin-3-galactoside (Cy-3-Ga), peonidin-3-galactoside (Pn-3-Ga), cyanidin-3-arabinoside (Cy-3-Ar), and peonidin-3-arabinoside (Pn-3-Ar), (18) and two minor anthocyanins: cyanidin-3-glucoside and peonidin-3-glucoside (5). Although quantitative methods for the determination of individual anthocyanins in cranberry have been described (1, 6, 9), information on variation in the proportions of anthocyanins in this fruit is limited. In a previous study (16), we reported small differences in the proportions of individual anthocyanins in the juice of dark- and light-colored subsamples of 45 cranberry clones. Subsequent investigations with highbush blueberries (11 cultivars) and thornless blackberries (33 cultivars) demonstrated large qualitative and quantitative differences among cultivars in the proportions of individual anthocyanins; the anthocyanin composition data falling into three distinct patterns for blueberry and five for blackberry (12, 14). Furthermore, the blackberry study showed large changes in the proportions of anthocyanins during ripening, reflecting differing rates of accumulation for the individual anthocyanins. In a recent study of factors affecting the total anthocyanin content of cranberries (13, 15), we observed extensive variability in the anthocyanin content of individual berries within samples, which we attributed to the superposition of environmental factors (i.e., light exposure and temperature) on ripeness differences. The occurrence of large differences in the proportions of individual anthocyanins in cranberry fruit might be expected to affect the color stability of cranberry products such as juice and sauce (4, 17). Also, extensive variation in the anthocyanin composition of cranberries might complicate the detection of adulteration in cranberry products (3). Therefore, it was of interest to us in the present study to determine the extent of variation in anthocyanin composition in cranberry fruit due to cultivar and differences in color development.

Materials and Methods

Determination of anthocyanin composition—1984 study. Samples of 'Franklin', 'McFarlin', and 'Searles' cranberries, weighing 3–4 kg each, were harvested from adjacent bogs at the Rutgers Univ. Blueberry and Cranberry Research Center in Chatsworth, N.J. in mid-Oct. 1984, frozen, and stored at −29°C for 6 months prior to analysis. While still frozen, the samples were sorted visually into light-, medium-, and dark-red subsamples. Duplicate 50-g portions of the frozen subsamples were homogenized in a semi-micro blending container and analyzed for soluble solids by refractometry (with correction to 20°) and for titratable acidity by titration with 0.1 N NaOH to a pH 8.1 end-point. The total anthocyanin content (TAcy) of fruit samples was determined by extraction of the homogenates with 95% ethanol : 1.5 M HC1 (85:15) and spectrophotometric analysis of the resulting extracts at 535 nm. Individual anthocyanins in cranberry samples were determined by HPLC analysis of the alcoholic extracts on an analytical reversed-phase C_{18} column with a concave binary gradient comprising 0.1 M phosphate buffer (pH 1.5) and acetonitrile. Pelargonidin was added to samples at a concentration of 200 ppm as the internal standard. Details of these procedures have been described previously (14, 16). Peak assignments were made on the basis of the known anthocyanin composition in cranberry and the expected order of elution of arabinosides and galactosides on a reversed-phase C_{18} column (1, 8); HPLC retention times of aglycons prepared by acid hydrolysis of isolated peaks (2), compared to retention times of cyanidin and peonidin standards; and the Euv, max : Evis, max and E_{440} : Evis, max ratios of the isolated peaks (2). The proportions of individual anthocyanins were calculated from their HPLC peak areas and the total peak area, excluding the area of the internal standard. To estimate the content of individual anthocyanins in cranberry samples, HPLC peak areas were normalized and multiplied by appropriate dilution factors. Anthocyanin contents were expressed as HPLC area units per gram of fruit, since extinction coefficients for cranberry anthocyanins dissolved in phosphate buffer–acetonitrile mixtures (the HPLC mobile phase) were not available. The combined effects of cultivar and subsample coloration and their interaction (nine treatments) on anthocyanin composition were determined by analysis of variance. The Bonferroni LSD was used to separate means (10).

To determine the relationship between the accumulation of individual anthocyanins and the state of the 1984 fruit samples, as indicated by their TAcy values and soluble solids : acidity ratios (SS:A), we plotted the content of individual anthocyanins in each subsample against the corresponding TAcy and SS:A

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Table 1. Composition and proportions of individual anthocyanins in cranberry cultivars, 1984.[z]

Cultivar	Subsample color	SS:A[y]	Total anthocyanin (mg/100 g)	Peak area percent				
				Peak 1 (Cy-3-Ga)	Peak 3 (Cy-3-Ar)	Peak 4 (Pn-3-Ga)	Peak 5 (unknown)	Peak 6 (Pn-3-Ar)
Franklin	Light red	3.5 e	34.4 f	25.1 abc	15.2 bc	33.1 cd	4.6 b	13.6 a
	Medium red	4.0 d	62.7 c	26.4 ab	14.4 bcd	38.8 ab	3.8 b	12.8 a
	Dark red	4.5 b	100.6 a	27.2 ab	13.5 cde	38.3 ab	3.6 b	12.3 a
McFarlin	Light red	3.4 e	25.3 g	25.1 abc	19.5 a	30.1 d	3.9 b	12.2 a
	Medium red	4.0 d	43.3 d	24.5 bc	16.6 b	35.4 bc	4.3 b	14.6 a
	Dark red	4.3 c	79.9 b	27.9 a	16.6 b	36.3 abc	3.2 b	13.1 a
Searles	Light red	4.3 c	25.5 g	17.3 d	10.8 e	39.6 ab	8.1 a	13.6 a
	Medium red	4.6 b	38.1 e	23.2 c	11.6 de	40.5 a	5.2 ab	13.0 a
	Dark red	5.0 a	61.3 c	24.4 bc	12.0 de	39.9 ab	5.3 ab	12.7 a

[z]Mean separation in columns by Bonferroni t test at $P < 0.05$.
[y]Soluble solids (percentage at 20°C) ÷ titratable acidity (percent citric).

Table 2. Correlation of anthocyanin (TAcy) and soluble solids-acidity ratio (SS:A), 1984.

Correlation	Anthocyanin peak[z]	Slope			Correlation coefficient		
		Franklin	McFarlin	Searles	Franklin	McFarlin	Searles
Anthocyanin accumulation vs. TAcy	1	0.31	0.36	0.34	0.998	0.990	0.989
	3	0.14	0.19	0.16	0.996	0.988	0.991
	4	0.45	0.47	0.50	0.993	0.998	0.988
	5	0.04	0.04	0.06	0.989	0.985	0.962
	6	0.13	0.16	0.16	0.993	0.999	0.987
Anthocyanin accumulation vs. SS:A	1	20.3	NL[y]	18.4	0.998	NL	0.996
	3	9.1	NL	8.5	0.996	NL	0.997
	4	29.2	NL	27.2	0.994	NL	0.996
	5	2.3	2.1	2.9	0.988	0.939	0.942
	6	8.4	NL	8.5	0.993	NL	0.995

[z]See Table 1 for peak identifications.
[y]NL = nonlinear.

values. Since the resulting curves appeared to be linear for most samples, correlation coefficients and regression slopes for these relationships were compared.

Atypical anthocyanin patterns—1980 and 1981 studies. Anthocyanin composition data, obtained in 1980 for 43 clones from the Univ. of Massachusetts Cranberry Experiment Station in East Wareham and in 1981 for three clones from Chatsworth were examined for evidence of atypical anthocyanin patterns. These data were obtained by HPLC analysis of expressed juice instead of alcoholic extracts, on a 30-cm μBondapak (Waters Associates, Milford, Mass.) C_{18} column with 81.7 water, 8.4 acetonitrile, 8.4 acetic acid, 1.5 phosphoric acid (by volume) as the mobile phase (isocratic). The samples and procedures are described in detail in a previous publication (16).

Samples having the highest peak area percentages for one or more individual anthocyanins were identified. Based on an assumption of normality, clones with atypical peak area values were compared against the rest of the sample population by calculating 99% confidence intervals for one atypical peak or 99% confidence ellipses for two atypical peaks.

Results and Discussion

Anthocyanin composition vs. cultivar and sample coloration. The cranberry samples compared in the 1984 study were selected because of the large differences in total anthocyanin content seen previously in samples of these cultivars (15). The data in Table 1 clearly show the greater amount of anthocyanin in 'Franklin' as compared to 'McFarlin' and 'Searles', as well as the greater anthocyanin content of the more highly colored subsamples, which were riper, as indicated by their greater SS:A values (13). The separation of cranberry anthocyanins and pelargonidin, the internal standard, by HPLC is illustrated by chromatograms obtained for light- and dark-colored 'Franklin' berries (Fig. 1). The major components, peaks 1, 3, 4, and 6, were determined to be Cy-3-Ga, Cy-3-Ar, Pn-3-Ga, and Pn-3-Ar, respectively, based on previously reported peak assignments (1, 16), HPLC retention time data for the aglycones, and spectral measurements reported previously (8).

With few exceptions, the proportions of individual anthocyanins in cranberry samples did not vary significantly with berry coloration or cultivar. In instances where significance was demonstrated, cultivar and coloration effects on anthocyanin composition were small. These results confirm our previous findings with cranberries from the 1980 and 1981 seasons that the relative proportions of individual anthocyanins for most clones varied within narrow limits and were similar for dark- and light-colored subsamples (16).

The similar anthocyanin patterns obtained with subsamples of different coloration suggest that the individual anthocyanins in cranberry fruit are each synthesized at rates that are constant relative to the overall rate of anthocyanin accumulation during color development. This constancy can be seen in the approximately linear relationships obtained between the individual anthocyanin contents (HPLC peak area per gram) and the total anthocyanin content of light-, medium-, and dark-red fruit sam-

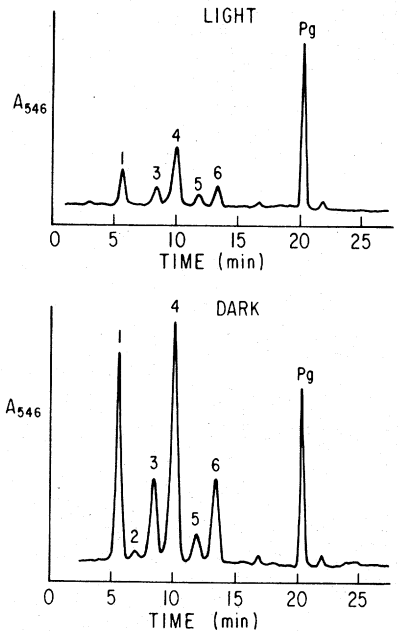

Fig. 1. HPLC chromatograms for light- and dark-red 'Franklin' cranberries. Peaks 1, 3, 4, and 6 are Cy-3-Ga, Cy-3-Ar, Pn-3-Ga, and Pn-3-Ar, respectively; Pg is pelargonidin, the internal standard.

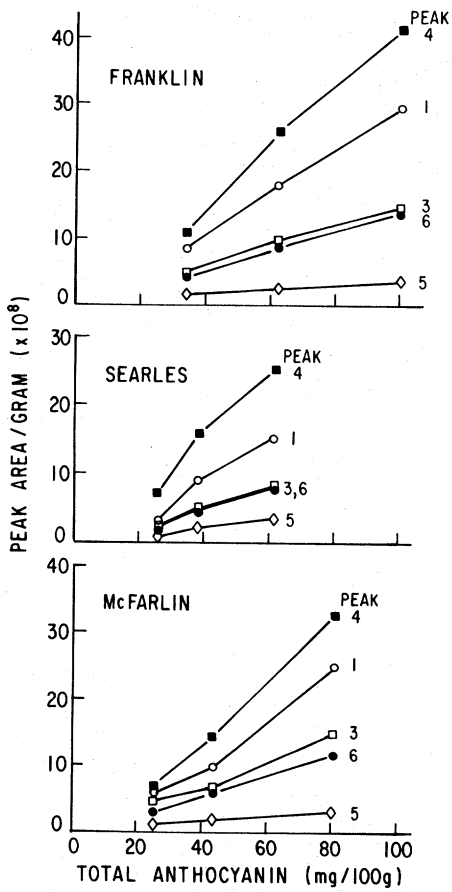

Fig. 2. Contents of individual anthocyanins (HPLC peak area per gram) vs. total anthocyanin content of light-, medium-, and dark-red subsamples of 'McFarlin', 'Franklin', and 'Searles' cranberries.

ples obtained in 1984 (Fig. 2). Slopes for corresponding peaks were similar for each of the three cultivars examined (Table 2). It would follow from the constant proportions of individual anthocyanins obtained during development that the rate-limiting step in anthocyanin biosynthesis precedes steps establishing the characteristic anthocyanin pattern in cranberry fruit, i.e., *O*-methylation (cyanidin vs. peonidin) and glycosylation (arabinosides vs. galactosides). Hydroxylation and subsequent methylation may occur at the cinnamic acid stage or later at the chalcone–flavonone stage in the biosynthetic pathway (7). Glycosylation is probably the last step in anthocyanin biosynthesis (7). According to Rhodes (11), enzymes catalyzing the first few steps in the pathway, from phenylalanine to the CoA thioesters of hydroxycinnamic acids (P.A.L., cinnamic acid 4-hydroxylase, and hydroxycinnamic acid CoA ligase), are most important in regulating the phenylpropanoid pathway.

We also obtained a linear relationship between the individual anthocyanin contents (HPLC peak area per gram) and values of SS:A for subsamples of 'Searles' and 'Franklin' but not 'McFarlin' cranberries, slopes for corresponding anthocyanins being similar for 'Searles' and 'Franklin'. Previously, we observed a linear relationship between concentrations of individual anthocyanins in thornless blackberry fruit and the SS:A ratio (14). The nonlinearity observed with 'McFarlin' cranberries may be an indication of the influence on anthocyanin biosynthesis of environmental factors such as light, superimposed on the ripening process (13).

Occurrence of atypical anthocyanin patterns. Most of the cranberry clones examined in the 1980 and 1981 studies were similar in anthocyanin composition (16). When anthocyanin composition data for clones giving the highest peak area percentage values were separated from data for the remaining clones, several apparent atypical patterns could be seen (Table 3). These were: 1) clones such as BD and No. 20 having a higher percentage of peak 1 (Cy-3-Ga) than the typical clones; 2) clones such as 'Early Black' and W41 that were high in peak 3 (Cy-3-Ar) as well as peak 1; and 3) clones such as 'Can Cp' and 'Wales Henry' that had higher proportions of peaks 4 (Pn-3-Ga) and 6 (Pn-3-Ar) than the other clones. However, when the significance of differences between typical and atypical populations was tested, clones with high levels of peaks 1 and 3, or 1 alone, were found to be within the 99% confidence intervals for the typical clones. Only 'Can Cp' and 'Wales Henry' showed evidence of a significant difference, falling just outside the 99% confidence ellipse calculated for peaks 4 and 6 with peak area percentages of 45.6 and 46.4 for peak 4 and 19.9 and 19.1 for peak 6, respectively. Therefore, evidence for the existence of atypical anthocyanin patterns in cranberry fruit is marginal at best and limited to only two clones with high proportions of peaks 4 and 6. This result is in marked contrast to our observations with blueberries (12) and blackberries (14), where clear evidence of qualitatively different anthocyanin patterns (missing anthocyanins or very large differences in proportions) could be seen.

Whether the relatively minor differences in anthocyanin patterns seen with the 1980 samples are genetic or represent variability due to environmental or analytical factors is not clear. A comparison of these data with anthocyanin data for samples of cranberries representing a different production season (1981) and location (Chatsworth, N.J.) suggests that the patterns are consistent for the limited numbers of cultivars compared (Table 4). However, differences within cultivars for peaks 1, 3, and 6

Table 3. Atypical anthocyanin patterns in dark-red cranberry samples, 1980 season.

Atypical pattern	Peak[z]	Sample population	No. observations	Peak area percentage Mean	Range	Standard deviation
High Peak 1	1	Atypical[y]	24	29.5	27.6–31.3	1.1
		Typical[x]	152	24.8	18.4–31.2	3.0
High Peaks 1 and 3	1	Atypical[w]	10	30.1	29.2–31.3	0.7
		Typical[x]	166	25.2	18.4–31.2	3.1
	3	Atypical[w]	10	20.0	18.8–21.5	1.1
		Typical[x]	166	15.9	11.8–20.4	1.9
High Peaks 4 and 6	4	Atypical[v]	20	44.9	42.2–47.1	1.6
		Typical[x]	156	38.9	32.6–44.1	2.7
	6	Atypical[v]	20	18.7	14.9–20.8	1.6
		Typical[x]	156	15.4	11.7–20.7	2.0

[z]See Table 1 for peak identifications.
[y]'Early Black', W41, W93, BD, No. 20.
[x]All other clones.
[w]'Early Black', W41, W93.
[v]'Can Cp,' 'Early Red', 'Holliston', 'Matthews', 'Middleboro', 'Wales Henry'.

Table 4. Stability of anthocyanin patterns in dark-red cranberry samples.

Cultivar	Anthocyanin pattern	Season	Location[z]	Peak area percentage[y] Peak 1	Peak 3	Peak 4	Peak 5	Peak 6
Franklin	Typical	1980	E. Wareham-1	24.6	15.0	41.8	3.8	14.8
			E. Wareham-2	25.3	14.3	42.6	4.0	14.6
		1981	Chatsworth	26.0	12.7	42.7	4.1	12.8
Pilgrim	Typical	1980	E. Wareham-1	27.8	16.4	38.7	2.8	14.3
			E. Wareham-2	27.3	16.9	38.8	3.2	13.8
		1981	Chatsworth	29.9	14.8	39.8	2.8	11.6
Early Black	High 1 and 3	1980	E. Wareham-1	31.0	18.8	34.2	2.6	13.4
		1981	Chatsworth	33.7	16.1	35.4	3.3	10.9
LSD[x]				1.6	0.8	1.2	0.6	1.6

[z]Chatsworth cranberries sampled twice in 1980.
[y]See Table 1 for peak identifications.
[x]Bonferroni LSD at 0.05 level, based on at least 54 df (all dark subsamples in 1980, 1981, and 1984).

were as great as differences between typical and atypical patterns ('Franklin' or 'Pilgrim' vs. 'Early Black').

Our results suggest that because the relative proportions of anthocyanins in cranberries do not depend on fruit ripeness or cultivar (except for minor differences with several atypical cultivars and selections having little or no commercial importance), different sources of fruit could be processed interchangeably without affecting product hue or color stability. Of course, the raw material would have to be blended to compensate for variation in total anthocyanin content and pH, both of which influence color expression. Nevertheless, the ability of cranberry processors to use raw materials which represent different cultivars or degrees of color development may be a significant advantage.

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