

# Color and Composition of Highbush Blueberry Cultivars

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**Abstract.** The anthocyanin content of ripe berry samples of 11 cultivars of highbush blueberry (*Vaccinium corymbosum* L.) varied over a 3-fold range. HPLC separation of individual anthocyanins in blueberry samples revealed 3 distinct anthocyanin patterns. Visible absorption spectra of aqueous berry extracts reflected differences in anthocyanin concentration and pH, the latter especially being evident with the more acidic berries of 'Coville' and 'Elliott'. Tristimulus reflectance measurements made on whole berries correlated with visual assessment of waxy bloom but not with anthocyanin content, anthocyanin pattern, or juice pH. SEM examination revealed 2 different surface structures in samples exhibiting bloom. Tristimulus parameters for blueberry juice were dependent on anthocyanin concentration, pH, and the occurrence of browning, but not on the pattern of individual anthocyanins.

Highbush blueberry color, an important quality factor influencing fresh-market value and the suitability of the berries for processing, is a highly complex attribute affected by the total anthocyanin content (7, 19), the quantity and structure of surface wax (2), the pH (17), and perhaps the distribution of individual anthocyanins (6) and the formation of metal complexes of anthocyanins (17). These factors are subject to genetic (3, 5, 11, 15, 16) and environmental influences (1, 20) and depend on the degree of ripeness (4, 8).

We report herein a study of color differences among highbush blueberry cultivars as part of a broader investigation of the expression of genetically controlled quality attributes in small fruits. Our specific objective was to assess the relative importance of the aforementioned factors in determining blueberry color. The effects of cooking and freezing on the color of these cultivars will be addressed in a subsequent paper.

## Materials and Methods

**Sampling and colorimetry of blueberry cultivars.** We obtained samples of 11 highbush blueberry cultivars ('Berkeley', 'Bluetta', 'Bluecrop', 'Blueray', 'Burlington', 'Collins', 'Coville', 'Earliblue', 'Elliott', 'Jersey', and 'Weymouth') from plantings at the USDA, Rutgers University Blueberry and Cranberry Research Center in Chatsworth, N.J. Samples were taken for evaluation, once in 1981, twice in 1982 (1–2 weeks apart), and once in 1983. Sample ripeness was judged at the time of harvest on the basis of skin color, ease of detachment, and the flavor of representative berries. In addition to ripe berries, small samples of berries ranging in color from green to dark blue or black (no waxy bloom) were taken for microscopic examination.

Freshly received samples were washed, drain-dried on cloth towels, and examined visually for waxy blooms. Nondestructive reflectance measurements were made with a Gardner XL-23 tristimulus colorimeter, standardized with a blue tile ( $Y = 39.30$ ,  $X = 38.59$ , and  $Z = 65.41$ ). Cylindrical optical cells (57.1 mm I.D.  $\times$  40 mm height) were filled to overflowing with blueberries, and  $L$ ,  $a_L$ , and  $b_L$  readings were taken as is and after rotating the cell  $180^\circ\text{C}$ .

**Blueberry composition and spectrophotometry.** About 100 g of berries were homogenized for 2 min at high speed in a semi-micro, stainless steel blending container (250 ml capacity) on a Waring base, blending being facilitated by pressing the blueberries against the blender blades with a wooden plunger. The pH of the homogenate was measured with a Corning Model 130 pH meter, and the soluble solids content of juice separated from the homogenate was determined with a Bausch & Lomb Abbe—3L Refractometer cooled to  $20^\circ$  to  $22^\circ\text{C}$ . Titratable acidity, expressed as percentage of citric acid, was determined by titrating a 5-g aliquot of homogenate, diluted with 45 ml distilled  $\text{H}_2\text{O}$ , to a pH 8.1 endpoint with 0.1 N NaOH. A 5-g aliquot of blueberry homogenate was diluted with 95 ml distilled  $\text{H}_2\text{O}$  for spectrophotometric measurements. The resulting 1:20 dilution was clarified by the addition of 4 g of Celite Analytical Filter Aid, followed by filtration under suction through Whatman No. 2 filter paper. Each filtrate was adjusted to the pH value of the corresponding homogenate by dropwise addition of 0.5 N HCl. The visible absorption spectrum (650 to 400 nm) of pH-adjusted filtrates was measured in a 10-mm rectangular cuvet with a Perkin-Elmer Model 552 UV-visible spectrophotometer. The absorption maximum ( $\lambda_{\text{max}}$ ) was located from the recorded spectrum, and the absorbance at that wavelength ( $A_{\lambda_{\text{max}}}$ ) was measured. In addition, the absorbance at 440 nm, an indication of browning (13), was measured and the ratio  $A_{440}/A_{\lambda_{\text{max}}}$  calculated. Each filtrate was readjusted to a single pH value, 3.3, by the addition of 0.1 N NaOH or 0.5 N HCl, as required, and the spectral measurements were repeated to permit direct comparisons of the absorption spectra of the different blueberry samples. Total anthocyanin in blueberry samples was determined by the method of Deubert (9), as modified by Sapers et al. (23). Total antho-

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cyanin was expressed as the product of the absorbance at  $\lambda_{\max}$  (543 nm) and the dilution factor because values of the extinction coefficients for blueberry anthocyanins were not available.

**Juice preparation and colorimetry.** "Drip" samples (exudate collected from thawing berries previously subjected to freeze-thaw cycling) were obtained from the 1981 blueberry samples. The drip samples were similar in pH, soluble solids, and spectral characteristics to juice obtained with an Acme Juicerator or by filtering berry homogenates. Juice was obtained from the 1982 blueberry samples by blending 120–180 g of berries, as described previously, and then pressing the resulting homogenates in bags comprised of 4–6 layers of cheesecloth. The juice was clarified by adding 3% Celite Analytical Filter Aid and filtering under suction through Whatman No. 2 filter paper. Tristimulus reflectance and transmission methods were made on 25-ml juice aliquots before and after pH adjustments to 3.3, as described previously.

**HPLC of blueberry anthocyanins.** The distribution of individual anthocyanins in blueberry cultivars was determined by HPLC with a Waters Associates chromatographic system comprised of a Model U6K injector, 2 Model 6000A solvent pumps, a Model 660 solvent programmer, a Model 440 absorbance detector (monitoring at 546 and 280 nm), and a Hewlett-Packard Model 3390A reporting integrator. The pigments were extracted by blending 50 g of berries with 50 ml solvent [95% ethanol : 1.5 M HCl (85:15)] at high speed in a semimicro blending container. The homogenate, mixed with 4 g Celite Analytical Filter Aid, was transferred to a 9.0-cm Whatman No. 5 filter disk in a Buchner funnel, and clarified extract was collected under suction. The clarified extracts were filtered through a 0.2- $\mu\text{m}$  Millipore membrane filter. A 4- $\mu\text{l}$  sample was injected onto a Waters 3.9 mm  $\times$  30 cm  $\mu\text{Bondapak C}_{18}$  (10  $\mu\text{m}$ ) stainless steel column. The mobile phase comprised 0.1 M pH 1.5 phosphate buffer (solvent A) and acetonitrile (solvent B), with a gradient of 7% B to 25% B in 60 min, following solvent program 7 (slightly concave curve) of the solvent programmer; the flow rate was 1.8 ml/min. Peaks absorbing at 546 nm were integrated and the area percentages calculated. Separated pigments were not identified but were designated by number, i.e., the order of elution.

**Scanning electron microscopy.** Selected longitudinal strips (3  $\times$  10 mm) of blueberry skin were removed with a razor blade and laid flat in a desiccator for 24 hr to dry (2) for scanning

electron microscopy (SEM). Portions (3  $\times$  5 mm) then were mounted on copper stubs by means of silver paint and sputter-coated with about 15 nm of gold-palladium. Specimens were observed at 15 kV in a JEOL 50A scanning electron microscope. Each mounted specimen was scanned in its entirety to ensure that micrographs were representative of the surface morphology, which can vary greatly within short distances.

**Statistical analysis.** The data in Tables 1, 3, 4, and 6 were analyzed by analysis of variance to test the effects of cultivar and time of harvest. Comparisons between means were made by application of the Bonferroni least significant difference method (21). All statistical computations were performed with the Statistical Analysis System (SAS Institute, Inc., Cary, N.C.), General Linear Models Procedure.

## Results and Discussion

**Composition of blueberry cultivars (Table 1).** Mean values of the pH, titratable acidity, soluble solids, soluble solids:acidity ratio, and total anthocyanin content for highbush blueberry cultivars, harvested twice during the 1982 season, indicate that most samples were fully ripe (4, 26). First harvests of 'Berkeley', 'Bluetta', 'Collins', and 'Earliblue' were higher in acidity than were the 2nd harvests, although soluble solids:acidity ratios for the former samples were still within the ripe range (15–26). Both harvests of 'Elliott' and 'Coville' (as well as a 3rd harvest of 'Coville' taken 9 days after the 2nd harvest) were consistently high in acidity, the soluble solids:acidity ratio and total anthocyanin content remaining constant for successive harvests. Whether these cultivars are inherently high in acidity or for some reason failed to ripen fully is not clear. Samples of 'Elliott' taken during the 1983 season gave similar values of the soluble solids:acidity ratio (11.9) and total anthocyanin content (233) as 1982 samples. However, 'Coville' samples taken in 1983 appeared to be riper than were 1982 samples, giving a higher soluble solids:acidity ratio (22.0) but a similar total anthocyanin content (143). The 11 cultivars showed large differences in total anthocyanin content; 'Burlington' and 'Elliott' contained 2–3 times as much pigment as 'Bluecrop' or 'Collins'. Other cultivars fell between these extremes. Samples of the same cultivar harvested at 1–2 week intervals were similar in anthocyanin content. Analysis of variance demonstrated that 86% of the total variability in titratable acidity and 90% of the total variability in total anthocyanin could be explained by cultivar differences. The remaining

Table 1. Composition of highbush blueberry cultivars (1982 season).<sup>z</sup>

Cultivar	pH	Titratable acidity (% citric acid)	Soluble solids (%)	SS/A ratio <sup>y</sup>	Total anthocyanin <sup>x</sup>
Berkeley	3.43 ab	0.47 bc	13.7 abc	29.8 ab	151 c
Bluetta	3.26 ab	0.60 bc	11.4 d	20.6 abc	161 c
Bluecrop	3.33 ab	0.60 bc	12.2 d	20.5 abc	85 d
Blueray	3.27 ab	0.55 bc	11.4 d	21.1 abc	143 c
Burlington	3.38 ab	0.70 bc	14.3 a	21.5 abc	270 a
Collins	3.28 ab	0.54 bc	12.3 bcd	23.2 abc	119 cd
Coville	2.97 ab	0.84 b	12.6 bcd	15.0 bc	139 c
Earliblue	3.46 ab	0.42 c	12.3 cd	30.7 ab	137 c
Elliott	2.85 b	1.31 a	11.4 d	8.7 c	214 b
Jersey	3.49 a	0.40 c	13.8 ab	34.6 a	160 c
Weymouth	3.28 ab	0.49 bc	11.2 d	22.7 abc	142 c

<sup>z</sup>Means of 2 harvests, each sampled twice and analyzed in duplicate. Mean separation in columns by Bonferroni least significant difference method, 5% level.

<sup>y</sup>Soluble solids (%) / titratable acidity (%).

<sup>x</sup>Total anthocyanin =  $A_{543} \times$  dilution factor.

variability could be attributed to differences in ripeness between samples harvested on different dates and between replicates from the same harvest.

**HPLC peak patterns for blueberry cultivars.** Preliminary HPLC separations of blueberry anthocyanins carried out in 1981 revealed the presence of as many as 16 different pigments (or their degradation products) in blueberry extracts. Ballinger et al. (6) found 14 anthocyanins in 'Croatan', a highbush blueberry cultivar, while Francis et al. (12) detected over 15 anthocyanins in lowbush blueberries. The 11 cultivars compared in 1982 (Fig. 1, Table 2) could be classified into 3 distinct groups on the basis of their HPLC peak patterns: 1) 'Bluecrop', 'Blueray', 'Bluetta', 'Earliblue', and 'Weymouth' having moderate to large peaks corresponding to pigments 1, 2, 4, 5, 6, 7, 8, 9, and 10 as well as small peaks 11, 13, 14, and 16; 2) 'Collins' and 'Jersey' resembling the first group but lacking peaks 11, 13, 14, 15, and 16; and 3) 'Berkeley', 'Burlington', 'Coville', and 'Elliott' resembling group 2, but being high in peak 8, deficient in peaks 2, 6, and 9, and having small peaks 3 and 15. Quantitative differences in anthocyanin distribution within groups were small and not related to the date of harvest. The significance of these results with respect to berry color is not clear. Absorption maxima for the individual anthocyanins found in lowbush blueberries, which also have been found in highbush blueberries (3, 5), do differ significantly (520–535 nm in acidic methanol) (12). However, among the samples examined, the pattern of major HPLC peaks is similar with no one component dominating (except perhaps for peak 8 in group 3), so that differences among anthocyanins in colorant properties might not be apparent. Parenthetically, the greater acidity of the 'Coville' and 'Elliott' samples in group 3 would be expected to enhance the color strength of their anthocyanins.

**Spectral properties of extracts from blueberry cultivars.** The visible absorption spectra of aqueous blueberry extracts exhibited an absorption band with values of  $\lambda_{max}$  between 519 and 525 nm, a manifestation of the extracted anthocyanins (Table 3). The location of absorption maxima did not appear to be strongly pH-dependent. However, pH had a large effect on anthocyanin absorbance, as can be seen by differences in the total absorbance value ( $A_{\lambda_{max}} \times$  dilution factor) between samples (see 'Coville' and 'Elliott') analyzed at their original pH and at pH 3.3. The former values would be an indication of the strength of color

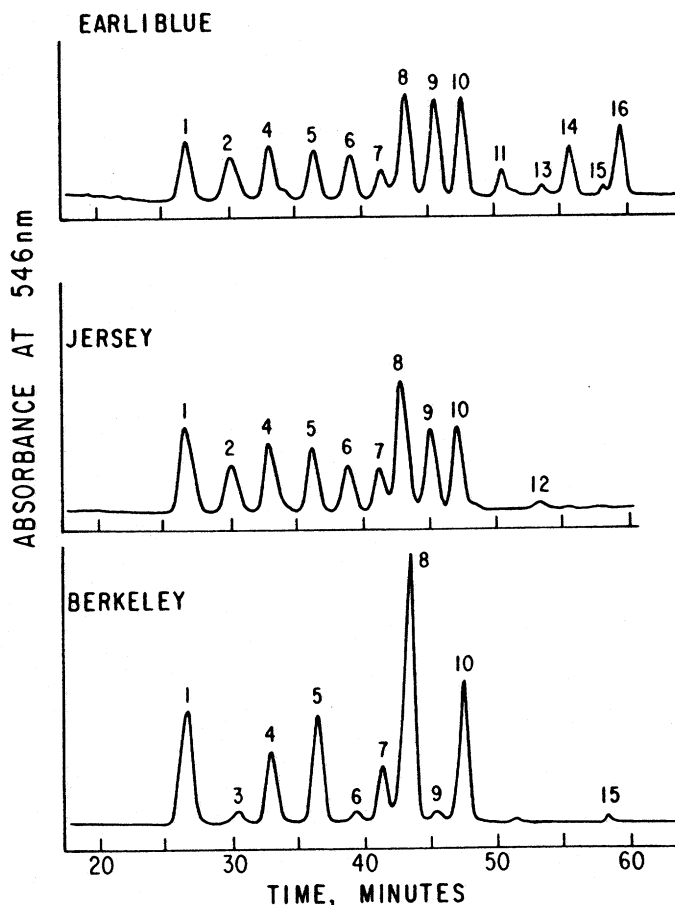


Fig. 1. HPLC of anthocyanins from 'Earliblue', 'Jersey', and 'Berkeley' blueberries.

expression by anthocyanins in blueberry juice, drip resulting from improper freezing and thawing of blueberries, and cooking water containing leached solids. For these cases, 'Elliott' would appear more intensely colored than the other cultivars, by a factor of 6 when compared to 'Bluecrop', the cultivar containing the least pigment. The total absorbance of extracts at pH 3.3 was correlated with the total anthocyanin content of the fruit ( $r = 0.73$ ). Variability in this relationship may be attributed to sample

Table 2. Distribution of anthocyanins in highbush blueberry cultivars (1982 season).

Cultivar	Mean HPLC peak area percentage <sup>a</sup>															
	Peak no.															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Bluecrop	8.5	5.3	---	8.2	6.1	4.6	5.2	21.8	11.8	18.2	1.4	---	1.6	3.5	---	3.7
Blueray	7.4	4.5	---	6.2	7.0	4.6	3.8	24.5	10.6	17.4	1.8	---	1.7	5.0	0.3	5.2
Bluetta	7.9	7.9	---	7.8	5.6	6.9	4.0	14.2	16.6	12.2	3.0	---	1.4	4.5	---	7.8
Earliblue	10.4	9.2	---	9.0	7.0	6.6	3.6	13.8	12.8	12.3	3.3	---	1.0	4.1	0.8	6.4
Weymouth	9.5	8.3	---	7.6	6.7	6.6	3.2	20.0	18.0	15.8	0.9	---	0.4	0.8	0.4	1.4
Collins	12.2	8.6	---	9.3	9.3	7.2	4.7	21.7	14.2	12.4	---	---	---	---	---	---
Jersey	12.4	7.6	---	9.6	8.8	6.3	5.6	21.6	12.9	13.8	---	1.0	---	---	---	---
Berkeley	16.2	---	1.8	9.4	12.8	1.2	6.2	33.2	1.2	17.1	---	---	---	---	0.5	---
Burlington	28.8	---	1.9	12.0	14.4	1.1	6.5	26.7	0.9	13.1	---	---	---	---	---	---
Coville	14.8	---	2.6	9.7	13.4	1.8	6.3	32.0	1.3	17.6	---	---	---	---	0.4	---
Elliott	20.0	---	2.1	11.2	12.8	1.1	5.8	30.4	1.2	14.9	---	---	---	---	0.5	---

<sup>a</sup>For HPLC—separated pigments detected at 546 nm and designated as peaks 1–16.

Table 3. Spectral characteristics of aqueous extracts from highbush blueberry cultivars.<sup>z</sup>

Cultivar	Extract pH	$\lambda_{max}$ (nm)	$A_{\lambda_{max}} \times \text{dilution factor}^y$		$A_{440}/A_{\lambda_{max}}^y$ at extract pH
			At extract pH	At pH 3.3	
Berkeley	3.4–3.6	521	11.3 b	13.0 ab	0.61 ab
Bluetta	3.2–3.4	521–522	16.8 ab	16.9 a	0.52 b
Bluecrop	3.2–3.4	520–523	5.3 b	5.4 b	0.90 a
Blueray	3.1–3.4	520–523	10.1 b	10.2 ab	0.63 ab
Burlington	3.3–3.7	522–524	16.5 ab	20.2 a	0.61 ab
Collins	3.3–3.5	520–522	9.8 b	10.6 ab	0.64 ab
Coville	2.9–3.1	520–521	18.2 ab	11.9 ab	0.49 b
Earliblue	3.2–3.8	520–525	10.8 b	11.5 ab	0.69 ab
Elliott	2.8–3.0	519–521	29.0 a	16.9 a	0.40 b
Jersey	3.4–3.5	522–523	10.6 b	12.7 ab	0.68 ab
Weymouth	3.3–3.6	521–523	12.4 ab	13.3 ab	0.61 ab

<sup>z</sup>Two harvests, each sampled twice and analyzed in duplicate.

<sup>y</sup>Mean separation in columns by Bonferroni least significant difference method, 5% level.

heterogeneity (berry ripeness and perhaps condition) and to variability in extraction efficiency during blending.

A potentially important difference among blueberry cultivars became evident when the spectra of extracts of 'Bluecrop' blueberries was examined. This cultivar, unlike the others, showed a pronounced tendency to undergo browning. Visible absorption spectra of 'Bluecrop' extracts showed an elevation of the "valley" at wavelengths less than the absorption maximum, a change that could be followed by measuring the ratio of  $A_{440}/A_{\lambda_{max}}$ . This characteristic of 'Bluecrop' may affect its suitability for processing unless the berries are blanched to inactivate polyphenol oxidase at the beginning of the process.

→ **Tristimulus colorimetry and waxy bloom of blueberries.** Tristimulus reflectance data can be used to characterize the colorant properties of a sample. Such measurements made on fresh blueberries (Table 4) showed a close relationship between the "L" value (higher L-values indicating lighter-colored samples) and visual assessments of waxy bloom. Regression analysis of this relationship yielded a correlation coefficient of  $r = 0.75$  when numerical scores were assigned to bloom (by the number of + shown in Table 4). Samples that scored high for bloom also tended to have slightly higher negative values of  $b_L$  (an indication of greater blueness), the correlation coefficient being  $r = -0.62$ .

The light-scattering property of surface wax responsible for its effect on blueberry color can be understood better by examination of the berry skin using SEM. SEM revealed the presence of 2 characteristic surface structures with ripe berries that exhibited waxy bloom. 'Blueray' and 'Burlington' had upright and flat wax platelets over a layer of continuous wax or annealed patches of wax (Fig. 2a and 2b). 'Elliott', also exhibiting bloom, showed few if any platelets but instead had an extreme degree of patchiness (Fig. 2c). The wax surface of ripe, black-colored blueberries appeared to be continuous or to consist of large annealed or overlapping plates without upright platelets or patchiness (Fig. 2d). Albrigo et al. (2) reported that the waxy bloom of a native blueberry, *V. elliotii* Chapm., was due to upright rods and platelets, seen in blue-colored fruit and absent in mature

black fruit. They reported that blue fruit contained more wax than did the black fruit and that  $\beta$ -diketone waxes were responsible for the upright wax forms found in the former. They concluded that wax metabolism in this species was under multiple gene control and that wax form should be an important consideration in breeding programs because of its involvement in blueberry color as well as in weight loss.

Table 4. Bloom and reflectance of highbush blueberry cultivars (1982 season).<sup>z</sup>

Cultivar	Bloom <sup>y</sup>	Reflectance			
		L	$a_L$	$b_L$	$\theta^*$
Berkeley	++++	22.9 a	1.3 a	-5.3 b	284 a
Bluetta	ND	19.0 ab	1.7 a	-4.4 ab	291 a
Bluecrop	++++	21.0 ab	1.4 a	-4.9 ab	285 a
Blueray	++	18.6 ab	1.9 a	-3.9 ab	296 a
Burlington	+++	20.6 ab	1.4 a	-4.6 ab	286 a
Collins	++	18.5 ab	1.5 a	-4.1 ab	291 a
Coville	++	18.9 ab	1.5 a	-4.2 ab	290 a
Earliblue	ND	16.9 b	1.8 a	-3.6 a	296 a
Elliott	++	19.7 ab	1.4 a	-4.3 ab	288 a
Jersey	+	18.7 ab	1.6 a	-4.2 ab	291 a
Weymouth	ND	16.5 b	1.7 a	-3.5 a	296 a

<sup>z</sup>Means of 2 harvests, each sampled twice and analyzed in duplicate. Mean separation in columns by Bonferroni least significant difference method, 5% level.

<sup>y</sup>Evaluated subjectively: + + + +, very extensive; + + +, extensive; + +, moderate; +, slight; ND, not determined.

<sup>\*</sup>Hue angle  $\theta = \tan^{-1}(b_L/a_L)$ .

Values of the hue angle  $\theta$  ( $\theta = \tan^{-1} b_L/a_L$ ), a measure of sample color, ranged between 282 and 302 for individual samples of the 11 cultivars examined; however, visual color differences between samples of similar bloom were not observed, and mean values of  $\theta$  for the 11 cultivars were not significantly different (Table 4). A hue angle of 270° would correspond to a blue sample while an angle of 360° (0°) would correspond to a red sample. The tristimulus parameters and visual appearance of berries were not affected by the harvest date, total anthocyanin content, pattern of individual anthocyanins,  $\lambda_{max}$ , or pH of the blueberry samples.

**Tristimulus colorimetry of blueberry juice.** Tristimulus colorimetry also was used to characterize the color of juice from blueberry samples. Visually, the samples varied in color from purplish-red to red. A comparison of tristimulus parameters for the different cultivars revealed extensive variability within and between samples, probably due to variability in the sample anthocyanin content and in the efficiency of pigment extraction during juice preparation (22). The effects of anthocyanin concentration and pH on tristimulus measurements were clarified with "drip" samples obtained from 'Collins' berries that had been diluted with H<sub>2</sub>O and adjusted to pH 3.0, 3.3, and 3.5



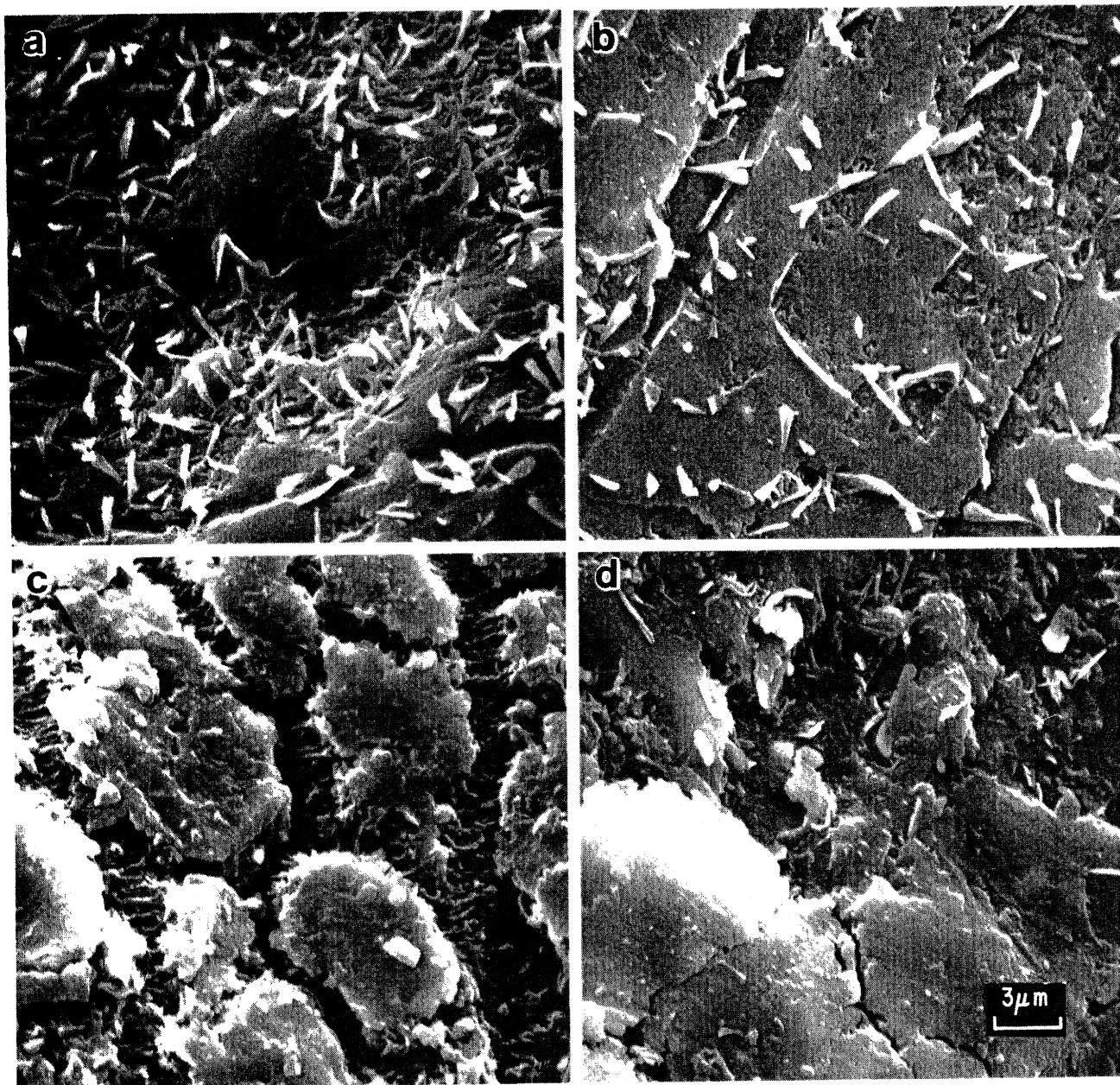


Fig. 2. Surface wax structures observed by SEM at  $3000\times$  for ripe blueberry cultivars: (a–c) ‘Blueray’, ‘Burlington’, and ‘Elliott’ with waxy bloom; (d) mature, black ‘Burlington’.

(Table 5). It is evident that reflectance and transmission  $L$  and  $\theta$  values both were related inversely to the anthocyanin concentration. Adjusting the pH above 3.3 increased reflectance  $L$  and decreased reflectance  $\theta$ , especially in more concentrated solutions; pH had little effect on transmission tristimulus values.

The reflectance  $\theta$  increased to a maximum value and then decreased over a narrow range of  $L$  when  $\theta$  values for juice samples were plotted against  $L$  (Fig. 3). Transmission  $\theta$ , however, increased gradually and then became constant with increasing  $L$ . Tristimulus maxima, which have been reported previously for other colorants (24, 25), are artifacts resulting from deficiencies in the equations used to compute  $a_L$  and  $b_L$  values from the X Y Z color scale (10). As such, they produce “areas of confusion” where color scale readings do not correlate with visual observations or pigment concentrations. Differences between samples must be interpreted with caution since tristim-

ulus data for undiluted blueberry juice samples fall within the “area of confusion.” However, the fact that the tristimulus data could be fitted to single transmission or reflectance  $\theta$  vs.  $L$  curves, irrespective of cultivar, suggests that the color of undiluted juice is not determined by cultivar. Rather, variation in juice color may result from differences in composition common to all cultivars, i.e., total anthocyanin content and perhaps acidity.

Some cultivar-related color differences may be seen with diluted blueberry juice. Tristimulus transmission measurements were made on filtered aqueous extracts of berry homogenates (prepared from frozen samples) that had been standardized by dilution to  $A_{\lambda_{\max}} = 1.0$  and adjustment of the pH to 3.3, to compensate for sample variability due to juice preparation and to avoid errors associated with the “area of confusion.”  $L$ -values for these samples varied within narrow limits (52.2–62.8), in-

Table 5. Effect of pH and dilution on tristimulus parameters of "drip" from 'Collins' blueberries (1981 season).

Dilution	pH	Reflectance		Transmission	
		L	$\theta$	L	$\theta$
None	3.0	4.1	5.3	14.8	8.0
	3.3	4.2	6.0	14.4	7.8
	3.5	5.1	2.7	14.0	7.6
1:1.5	3.0	4.9	10.3	20.6	11.3
	3.3	5.0	10.5	20.8	11.5
	3.5	5.7	8.1	20.6	11.3
1:2	3.0	5.8	13.3	25.6	13.4
	3.3	6.1	12.6	26.5	13.4
	3.5	6.8	10.4	26.9	13.4
1:2.5	3.0	6.7	13.1	30.0	14.5
	3.3	7.1	11.6	31.7	14.4
	3.5	7.9	9.9	32.8	14.7

dicating that the standardized solutions were similar in lightness. Values of the hue angle (Table 6) varied from 4.2 for 'Elliott' to 17.0 for a 'Bluecrop' sample, the range for most cultivars (2 harvests, each sampled twice) being relatively narrow. Color differences between standardized extracts of 'Burlington' ( $\theta = 10.0$ ), 'Bluecrop' ( $\theta = 8.8$ ), and 'Bluetta' ( $\theta = 6.2$ ) were detected with difficulty when examined initially by 4 observers; 'Bluetta' appeared slightly more pink than did the other samples. However, after being held for one hr at room temperature, 'Bluecrop' developed a more orange-red color and increased in hue angle to 15.6 while the other extracts were unchanged relatively in color and hue angle ('Burlington', 11.5 and 'Bluetta', 6.3).

No relationship was seen between the hue angles calculated for each cultivar and the pattern of anthocyanins for that cultivar (Table 2). The hue angle, however, was correlated highly with the ratio of  $A_{440}/A_{\lambda_{max}}$  ( $r = 0.95$ ) (Fig. 4). A high value of this ratio previously was associated with browning in extracts of fresh 'Bluecrop' berries. While no difference was seen between ratios obtained for extracts of fresh and thawed-frozen berries, it is possible that enzymatic browning occurred during the preparation of berry homogenates prior to extraction with both types of samples, thereby shifting the color towards a more orange hue of red. Chandler and Highlands (7) reported color differences between juice pressed from uncooked blueberry pulp

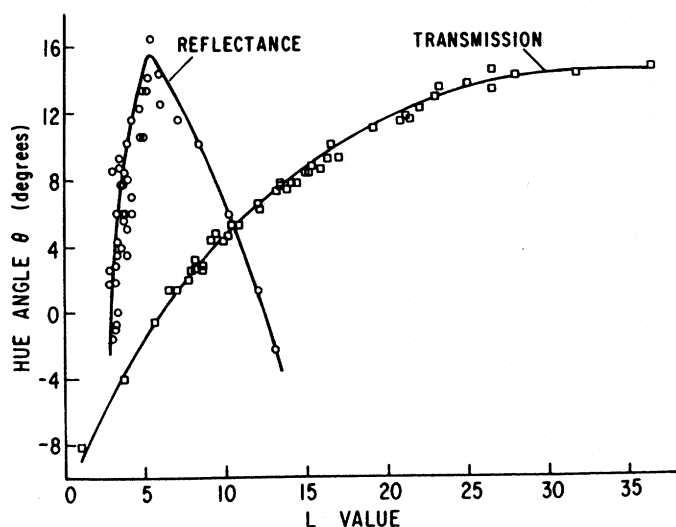


Fig 3. Effect of L-value on hue angle  $\theta$ , measured in reflectance and transmission modes, for juice of highbush blueberry cultivars.

Table 6. Tristimulus transmission hue angle for diluted blueberry extract, standardized at  $A_{\lambda_{max}} = 1.0$  and pH 3.3.

Cultivar	No. of samples <sup>z</sup>	Hue angle	
		Range	Mean <sup>y</sup>
Berkeley	4	10.4-12.7	11.8 bc
Bluetta	4	5.4- 7.7	6.4 e
Bluecrop	4	12.4-17.0	14.1 a
Blueray	4	8.0-10.2	9.2 d
Burlington	2	12.6-13.0	12.8 ab
Collins	4	10.2-13.6	12.3 ab
Coville	6	7.8-11.0	9.6 d
Earliblue	4	9.4-11.8	10.3 cd
Elliott	4	4.2- 5.1	4.8 e
Jersey	4	8.6-11.0	10.1 cd
Weymouth	4	8.5-11.3	9.8 cd

<sup>z</sup>Two harvests, each sampled twice.

<sup>y</sup>Mean separation by Bonferroni least significant difference method, 5% level.

following a pectinol treatment at 18° to 21°C and juice pressed from pulp heated to 63°, the former being red and the latter deep violet or purple. Fuleki and Hope (14), in a later study, obtained spectral data for similarly treated juice samples, showing higher values of the ratio  $A_{430}/A_{520}$  in cold press pectinol-treated juice prepared from frozen, thawed blueberries ( $A_{430}/A_{520} = 0.74$ ) than in hot press juice ( $A_{430}/A_{520} = 0.52$ ). We speculate that this difference resulted from enzymatic browning during thawing and/or pectinol treatment which was precluded in the hot press samples by inactivation of the enzymes involved.

The higher hue angle obtained with 'Bluecrop' extract, to some extent, may reflect its low anthocyanin content relative to the content of flavonols or other yellow pigments. Some evidence for this is seen in the spectral data obtained with ethanol extracts prepared for the determination of total anthocyanin under conditions precluding enzymatic browning. The ratio of the absorbance at 332 nm [a shoulder of the blueberry UV spectrum in a wavelength range attributed to flavonol absorption (18)] to the absorbance at 543 nm, the anthocyanin maximum, varied between 0.64 and 0.72 for 'Bluecrop'. In contrast, the same ratio varied between 0.23 and 0.27 for 'Bluetta' and between

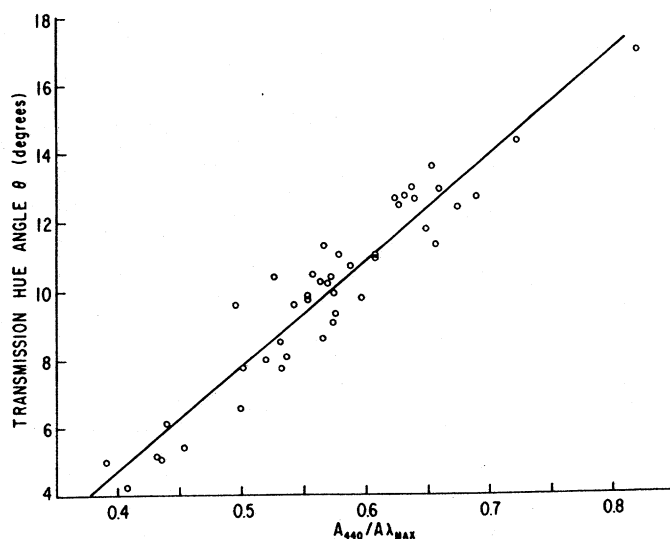


Fig. 4. Relationship between transmission hue angle  $\theta$  and the ratio  $A_{440}/A_{\lambda_{max}}$  for blueberry juice standardized by dilution to  $A_{\lambda_{max}} = 1.0$  and adjustment to pH 3.3.

0.25 and 0.39 for 'Elliott'. The lack of a consistent relationship between this ratio and the hue angle for other cultivars, however, suggests that the proportion of yellow pigments to anthocyanins is not a sufficient explanation of color variation in the juice of blueberry cultivars.

### Conclusions

Highbush blueberry cultivars differ significantly in acidity and total anthocyanin content. The distribution of HPLC peaks, presumed to be individual anthocyanins or their degradation products, also varies, falling into 3 distinct patterns for the 11 cultivars compared.

Color expression in fresh blueberries is determined primarily by the extent of waxy bloom rather than by the total anthocyanin content, distribution of individual anthocyanins, or pH. Cultivars differ in the surface structure of wax responsible for bloom.

Color expression in aqueous blueberry extracts and juice depends on the anthocyanin concentration and pH and is influenced by the tendency of cultivars such as 'Bluecrop' to undergo browning reactions. The hue of blueberry juice is not related to cultivar differences in the pattern of individual anthocyanins.

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