

# Anthocyanin Distribution in 'Delicious' Apples and the Relationship between Anthocyanin Concentration and Chromaticity Values

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**Abstract.** Fruit of 10 'Delicious' apple (*Malus domestica* Borkh.) strains were harvested 149 days after full bloom in 1988. Fruit color was measured at four locations on each fruit at the midpoint between the stem and calyx end with a Minolta CR-200b portable tristimulus calorimeter. Anthocyanin content of corresponding skin disks was determined spectrophotometrically. Significant differences existed among strains in both the amount and distribution of anthocyanin around the fruit. High-coloring strains had a significantly higher anthocyanin concentration at both the blushed and the nonblushed surface when compared to low-coloring strains. A linear regression of anthocyanin content on the ratio of  $(a^*/b^*)^2$  provided an  $R^2 = 0.59$ ; precision was enhanced by using a separate equation for each strain ( $R^2 = 0.80$ ). Regressing  $\log(\text{anthocyanin})$  on  $L^*$  using two linear splines yielded an  $R^2 = 0.78$ . These relationships allow the use of a portable calorimeter for rapid, nondestructive estimation of fruit anthocyanin content in situ.

Fruit color, an important marketing attribute of 'Delicious' apples, influences both consumer acceptance and sales (Smith and Frye, 1964). New strains of 'Delicious' have been introduced based on their superior coloration (Fisher and Ketchie, 1989). Surface color is also an important criterion in establishing U.S. grade standards for 'Delicious' apples (U.S. Dept. of Agr., 1972), and poor coloration is an important cause for reduction in grade (Baugher et al., 1990).

Color development in fruit is influenced by cultural and environmental factors. Two environmental factors that are important for red pigment development of fruit are temperature (Creasy, 1969) and light (Heinicke, 1966; Proctor and Creasy, 1971). Although temperature cannot be readily altered under field conditions, light penetration into the canopy can be enhanced by using a dwarfing rootstock and by proper training and pruning (Heinicke, 1975). However, inherent variations in the environment result in strains that ordinarily would develop good color but fail to do so under an elevated temperature or a low-light environment. Furthermore, high-density training systems, such as the Lincoln canopy, can cause shading and result in poor color development, especially under the environmental conditions of the eastern United States (Blizzard et al., 1988), and summer pruning may not rectify this problem (Keiser, 1988).

Although selecting strains with high coloring potential is important, it is equally important to determine the uniformity in fruit coloration in each strain. Strains that have a high anthocyanin level on the side not exposed to light would produce fruit having improved color under low-light or shaded conditions.

Changes in anthocyanin level in fruit have been measured during the growing season (Creasy, 1969; Murphey and Dilley, 1988). The procedure involves extracting anthocyanin from the fruit skin and measuring it spectrophotometrically. This procedure, although accurate, is both time-consuming and destructive. Rapid, nondestructive methods for estimating fruit anthocyanin levels would make it possible to follow changes in

individual fruit during the growing season or following the effects of various treatments.

The objectives of this study were to determine the uniformity of anthocyanin levels in fruit of 10 'Delicious' apple strains and to relate anthocyanin concentration to instrumentally measured fruit chromaticity values.

## Materials and Methods

Fruit were obtained from the 'Delicious' apple strain evaluation block at the West Virginia Univ. Farm, Kearneysville. Trees spaced  $4.9 \times 6.1$  m were planted in north-south-oriented rows on Hagerstown silt loam soil during 1981 and 1982. A ground cover of Kentucky-31 fescue was established between the rows immediately after tree planting. Recommended herbicide and pesticide programs were followed, and all trees received uniform annual fertilizer application. Trees of all strains were trained to a modified central leader by pruning during the dormant season.

On 21 Sept. 1988 (149 days after full bloom), five fruit were harvested from the outer periphery of two trees of each of the 10 strains. A single harvest date was used because results from a 1988 study on in situ color development of 'Delicious' strains showed that strains approach their ultimate color by the end of the first week of September (Singha et al., 1989). Immediately after harvest, fruit were gently wiped with a soft cloth to remove any dust or spray residues and fruit color was measured at four locations  $90^\circ$  apart on each fruit: the blushed surface exposed to sunlight, the nonblushed surface, and two intermediate points between the blushed and nonblushed surfaces. Color measurements were made by placing the 8-mm-diameter measuring area of a Minolta CR-200b portable tristimulus calorimeter (Minolta, Ramsey, N. J.) at the midpoint between the stem and calyx end of each measuring location and recording fruit chromaticity in Commission International d'Eclairage  $L^*$ ,  $a^*$ , and  $b^*$  color space coordinates (Hunter, 1975). The meter was calibrated at illuminant condition C (6774K) with a white standard before use.

Following color measurement, fruit were stored at  $-20^\circ\text{C}$ . Anthocyanin concentration was determined immediately after removing the fruit from storage. Individual skin disks, 10 mm in diameter, corresponding to each location of color measurement were extracted for 2 h in 1.5 ml of methyl alcohol con-

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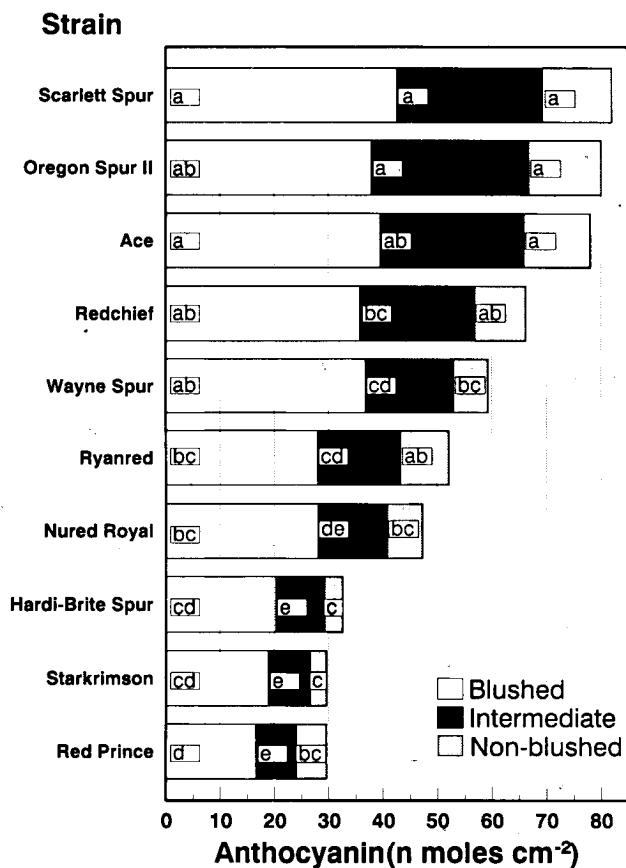


Fig. 1. Anthocyanin content of the blushed, nonblushed, and intermediate fruit surfaces of 'Delicious' apple strains. Mean separation within fruit surface between strains by Duncan's new multiple range test ( $P = 0.05$ ).

taining 1% HCl. Extraction was conducted in the dark at room temperature on a rotary shaker at 150 rpm. Absorbance of extracts was measured using a Perkin Elmer model 55 spectrophotometer (Perkin Elmer; Norwalk, Conn.). Anthocyanin absorbance was obtained from  $(A_{530} - A_{620}) - 0.1(A_{650} - A_{620})$ , and its concentration was determined using a molar extinction coefficient of  $4.62 \times 10^4$  (Zapsalis and Francis, 1965).

Anthocyanin content of the blushed, nonblushed, and the mean of two intermediate fruit surfaces was compared using Duncan's new multiple range test. The relationships of anthocyanin con-

centration on  $L^*$ ,  $a^*$ , and  $b^*$  were investigated using a variety of regression models both ignoring and including strain differences.

## Results and Discussion

**Anthocyanin distribution in fruit.** Differences existed between 'Delicious' strains in both the amount and distribution of anthocyanin around the fruit (Fig. 1). The anthocyanin content of the fruit skin of 'Scarlett Spur', 'Oregon Spur II', and 'Ace', which were selected for their superior coloration (Fisher and Ketchie, 1989), was more than double that of 'Hardi-Brite Spur', 'Starkrimson', and 'Red Prince'. These differences occur because the blushed, intermediate, and nonblushed surfaces of the poorly colored strains have a lower anthocyanin concentration.

There were no differences in the anthocyanin content of the blushed surfaces of 'Scarlett Spur', 'Oregon Spur II', 'Ace', 'Redchief (Campbell)', and 'Wayne Spur' (Fig. 1). However, the anthocyanin concentration of the intermediate surfaces of 'Scarlett Spur' and 'Oregon Spur II' was higher than that of 'Redchief' and 'Wayne Spur'. Although the blushed surface of these five strains has good coloration, the higher coloration of the fruit surface not directly exposed to sunlight would be an asset with 'Scarlett Spur', 'Oregon Spur II', and 'Ace' from both the standpoint of consumer appeal and U.S. grade standards.

Although the temperature at a given orchard site is critically important in color development (Creasy, 1956), 'Scarlett Spur', 'Oregon Spur II', and 'Ace' would be expected to produce better-colored fruit at any location than 'Starkrimson' and 'Hardi-Brite Spur'. Blizzard et al. (1988) reported poor color development in 'Topred' trained to the Lincoln canopy trellis system under the environmental conditions of the eastern United States. One approach to improving fruit color with this training system under these environmental conditions would be to select strains that have the potential to synthesize high levels of anthocyanin in a low-light environment. Consequently, using 'Scarlett Spur', 'Oregon Spur II', or 'Ace' should result in improved fruit color and a higher packout.

**Chromaticity values and their relationship to anthocyanin concentration.** The strains in this study were chosen based on variations in their coloration potential and, consequently, show wide variations in chromaticity values. In all strains, the blushed surface of the fruit was a significantly darker red (a lower  $L^*$  value) than the intermediate surface, and the nonblushed surface had the lightest coloration (Table 1). Comparisons among strains

Table 1. Chromaticity values of the blushed, nonblushed, and intermediate fruit surfaces of 'Delicious' apple strains.

Strain	$L^*$			$a^*$			$b^*$		
				Fruit surface					
	Blushed	Intermediate	Nonblushed	Blushed	Intermediate	Nonblushed	Blushed	Intermediate	Nonblushed
Ace	34.5 a	37.3 b	41.3 c	22.5 a	24.2 b	25.7 c	8.6 a	10.2 b	12.9 c
Hardi-Brite Spur	37.6 a	41.4 b	49.9 c	26.3 b	26.6 b	24.0 a	10.1 a	12.1 b	17.7 c
Nured Royal	36.6 a	39.5 b	43.1 c	25.7 a	27.9 b	29.3 b	10.6 a	12.6 b	15.5 c
Oregon Spur II	35.8 a	38.2 b	42.6 c	23.9 a	24.8 a	26.7 b	8.8 a	10.1 b	13.2 c
Redchief	33.7 a	36.6 b	41.7 c	22.2 a	24.7 b	27.1 c	7.6 a	9.7 b	13.3 c
Red Prince	40.4 a	44.9 b	49.4 c	26.7 b	26.6 b	24.5 a	12.3 a	15.5 b	18.4 c
Ryanred	36.7 a	40.7 b	44.6 c	22.0 a	23.5 b	22.9 a	9.4 a	12.3 b	15.6 c
Scarlett Spur	34.2 a	36.3 b	39.2 c	21.3 a	23.3 b	25.4 c	7.4 a	8.6 b	10.6 c
Starkrimson	35.9 a	41.2 b	48.9 c	24.3 a	26.0 a	25.5 a	9.2 a	12.8 b	18.0 c
Wayne Spur	35.9 a	40.0 b	46.3 c	24.9 a	26.5 a	25.2 a	9.1 a	11.7 b	15.3 c
SE	0.6	0.6	1.1	0.6	0.4	0.8	0.5	0.5	0.8

Mean separation within rows and chromaticity parameters by Duncan's new multiple range test ( $P = 0.05$ ).

Table 2. Coefficient of determination ( $R^2$ ) for selected regression models relating chromaticity values to anthocyanin content in fruit of 10 'Delicious' apple strains.

Variable	$R^2$
Simple linear models	
$a^*$	0.10
$b^*$	0.51
$a^*/b^*$	0.53
$L^*$	0.54
$\tan^{-1} b^*/a^*$ (hue angle)	0.34
$\sqrt{a^{*2} + b^{*2}}$ (chroma)	0.47
$(a^*/b^*)^2$	0.59
$(a^*/b^*)^2$ , with separate equation for each strain	0.80
More complex models	
$(a^*/b^*)^2$ , $L^*(a^*/b^*)^2$	0.73
$(a^*/b^*)^2$ , $L^*(a^*/b^*)^2$ , with separate intercept for each strain	0.78
$(a^*/b^*)^2$ , $L^*(a^*/b^*)^2$ , with separate equation for each strain	0.81

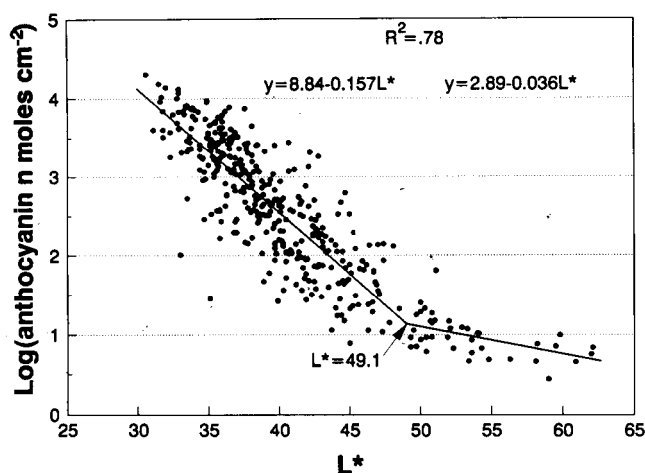


Fig. 2. Relationship between, extractable anthocyanin and  $L^*$  values measured at harvest in fruit of 10 'Delicious' apple strains.

indicate that the nonblushed surface of 'Hardi-Brite Spur', 'Red Prince', and 'Starkrimson' had the lightest fruit color. These strains also had a low anthocyanin content on the nonblushed surface (Fig. 1).

The  $a^*$  value (a measure of redness) tended to vary both among strains and fruit surfaces. In 'Starkrimson' and 'Wayne Spur', there were no differences in  $a^*$  values between the fruit surfaces, whereas in 'Hardi-Brite Spur' and 'Red Prince', the blushed and intermediate surfaces had a significantly higher  $a^*$  value than the nonblushed surface. In the high-coloring strains, including 'Oregon Spur II', 'Scarlett Spur', 'Ace', and 'Redchief', the nonblushed surface had higher  $a^*$  values than the blushed surface. Given that the blushed surface of these strains has a significantly higher anthocyanin content than the nonblushed surface implies that the  $a^*$  value per se is poorly related to anthocyanin concentration; this was validated statistically (Table 2). Further,  $a^*$  values were previously reported to be poorly related to fruit color ratings by a sensory evaluation panel (Singha et al., 1991). The  $b^*$  value (a measure of yellowness) in all strains was significantly higher on the nonblushed surface (Table 1). Furthermore, the three most poorly coloring strains ('Hardi-Brite Spur', 'Starkrimson', and 'Red Prince') had the highest  $b^*$  values for the nonblushed surface. These high  $b^*$  values

would be expected to influence the  $a^*/b^*$  ratio and, therefore, the appearance and visual rating of the fruit (Singha et al., 1991). The  $b^*$  value and, consequently, the  $a^*/b^*$  ratio relate far better to the extractable anthocyanin level than does the  $a^*$  value (Table 2).

A number of simple linear models used to relate chromaticity coordinates to anthocyanin content gave poor to satisfactory results (Table 2). The linear regression of the anthocyanin content on  $(a^*/b^*)^2$  for all strains provided an  $R^2$  of 0.59; precision was enhanced by using a separate equation for each strain ( $R^2 = 0.80$ ). Thus, similar to estimating chlorophyll concentration (Singha and Townsend, 1989),  $(a^*/b^*)^2$  maybe used for estimating anthocyanin concentration. However, the necessity of using a separate equation for each strain may create difficulties when comparing strains.

The effectiveness of using  $(a^*/b^*)^2$  may be improved by modifying the equation to include  $L^*$  (Table 2). The functional form of the more complex models in Table 2 may be written as  $(B_1 - B_2L^*)(a^*/b^*)^2$ . Thus, for fruit with light color (large  $L^*$ ); color changes are not as closely related to changes in anthocyanin as when the fruit become darker (smaller  $L^*$ ). This relationship is illustrated by the spline regression where the least-squares estimate of the intersection point for the two regressions is  $L^* = 49.1$  (Fig. 2). Consequently, color changes that occur before the fruit darken to  $L^* < 50$  must be due primarily to other pigments. Although the differences among the more complex models (Table 2) are statistically significant ( $P < 0.01$ ), the increased precision may not be of practical importance.

This research has established relationships between anthocyanin concentration and chromaticity values. The choice of whether a simple or complex model should be used will depend on the precision required for a particular investigation.

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