Leakage of Anthocyanins from Skin of Raw and Cooked Highbush Blueberries (*Vaccinium corymbosum* L.)

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ABSTRACT

Anthocyanin leakage from raw, dewaxed, or cooked blueberries was determined by spectrophotometric analysis of water in which berries were stirred. Leakage did not occur with fresh berries but was observed in samples refrigerated for 5 wk that contained soft berries. Dewaxing produced minimal leakage except with samples of poor condition. Pigment losses from dewaxed berries probably resulted from rupturing of the weakened skin. Leakage from cooked berries was more extensive, leakage vs stirring time curves being linear or two-phase linear. Leakage rates for cooked berries varied among the cultivars compared and appeared to be associated with the incidence of skin rupturing and with berry pigment content.

INTRODUCTION

THE LEAKAGE OF EXUDATE from the skins of fruits greatly influences product appearance and acceptability, especially if the exudate is pigmented. Dekazos and Smit (1976) attributed leakage of juice from fresh rabbiteye blueberries, a storage defect, to physiological breakdown, resulting from changes in the berry environment within closed containers. The loss of cellular fluids from thawing plant tissue (drip) depends on the method of freezing, the conditions and duration of frozen storage, the method of thawing, and the measurement technique (Joslyn, 1966). Previously, we observed cultivar-related differences in the tendency of highbush blueberries to leak during thawing and to impart color to water during cooking (Sapers et al., 1984b).

These phenomena are related to the integrity or permeability of the skin to cellular fluids and can be understood in terms of the barrier properties of the different structures comprising the skin, i.e., the outermost epicuticular wax layer, the underlying cuticle, and the epidermal and subepidermal cell layers in which the anthocyanin pigments are located. Schönherr (1976) found that the water permeability of isolated cuticular membranes was completely determined by the cuticular wax and was independent of membrane thickness. Norris (1974) reported that dewaxing greatly increased the permeability of isolated plant cuticles to 2,4-dichlorophenoxyacetic acid (2,4-D). Albrigo et al. (1980) observed that weight loss due to the dehydration of wild blueberries (*Vaccinium elliotti* Chapm.) was usually greater with black-colored berries than with blue-colored berries which have a higher wax content.

Our objective in this study was to relate anthocyanin leakage from the epidermal cells of blueberries during refrigerated storage and cooking to the barrier properties of the skin and its constituent parts in order to understand the mechanism of leakage and the physical basis of cultivar differences in leakage behavior. Leakage during thawing of frozen blueberries will be the subject of a separate report.

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MATERIALS & METHODS

Source and treatment of blueberries

Samples of seven highbush blueberry cultivars (Berkeley, Bluecrop, Burlington, Coville, Elliott, Jersey, and Weymouth) were obtained from the USDA, Rutgers University Blueberry and Cranberry Research Center in Chatsworth, NJ, in 1983. Cleaned, dry berries were packaged in 1/2-gallon polyethylene containers, covered with lids having 4 mm diam. holes at 3 – 4 cm intervals to permit gas exchange, and stored at 3°C for as long as 5 wk.

Anthocyanin leakage was determined with 50g portions of berries that had been sorted to exclude atypically large, small, or defective berries and then equilibrated at about 25°C for 1 hr prior to the determination. To determine the effects of cooking on anthocyanin leakage, 50g portions of refrigerated berries were placed in a small wire kitchen strainer, held over rapidly boiling water in a stainless steel beaker heated on a hot plate (covered with a metal lid), and steamed for exactly 1 min under standardized conditions. The steamed berries were cooled by immersion in ice water for 1 min and then rinsed with three successive 30 – 35 mL portions of distilled water in a 60 mL coarse porosity fritted glass filter funnel under suction to remove residual cooling water prior to the measurement of leakage.

To determine the effect of epicuticular wax on anthocyanin leakage, 50g portions of fresh berries, equilibrated to 25°C, were dewaxed by stirring for 1 min in 100 mL $CHCl_3$ under standardized conditions at ambient temperature. Dewaxed berries were separated from the $CHCL_3$ in a fitted glass filter funnel under sucion.

To determine the effect of berry condition on anthocyanin leakage, refrigerated samples were sorted prior to weighing to exclude berries with visible defects. Berries that were soft to touch but appeared sound in other respects were either included or rejected, depending on the purpose of the experiment.

Model system for evaluation of anthocyanin leakage

Following equilibrium to room temperature (and rinsing of steamed berries), each preweighed portion of berries was added to 500 mL distilled water in a 600 mL beaker containing a 50 mm magnetic stirring bar and was stirred under standardized conditions for 24 min. At 4 min intervals, approximately 10 mL aliquots were withdrawn with a wide tip serological pipet and filtered through Whatman No. 1 paper under suction to remove seeds, wax and other particulate matter released from the stirring berries. A 5 mL portion of filtrate was added to 5 mL pH 3 McIlvaine's buffer (Hodgman, 1954), and the remaining filtrate was returned to the sample. Following the 24 min stirring procedure, the number and condition of berries in the samples and the turbidity of the water were noted.

Anthocyanins in the diluted aliquots were estimated spectrophotometrically at 519 nm, the visible absorption maximum, with a Perkin-Elmer Model 552 UV-visible spectrophotometer. Since anthocyanin leakage usually was linear or two-phase linear with time, the rate of leakage was determined as the slope of the absorbance vs time curve, calculated by linear regression analysis. Leakage rates were determined with 4 – 5 replications.

Leakage rates were compared with berry total anthocyanin content, determined by spectrophotometric analysis of acidified ethanolic extracts of blueberry samples (Sapers et al., 1983); the soluble solids-titratable acidity ratio (SS/A) (Sapers et al., 1984a); and total surface area, estimated from the weight (W) and number of berries (N) in a sample, assuming the berries to be spherical and to have a density of 1.04 (corresponding to a 10% sugar solution): Total surface area (cm^2) = 4.71 $(W^2N)^{1/3}$.

Statistical methods

Comparisons of leakage rates between cultivars were made by an analysis of variance and application of Scheffe's test (Scheffe, 1953). Relationships between the leakage rates and various other parameters were investigated by means of correlation analysis.

RESULTS & DISCUSSION

Anthocyanin leakage from raw and dewaxed blueberries

Raw highbush blueberries from fresh samples showed no significant anthocyanin leakage when tested in our model system, as would be expected from common experience (Fig. 1, Bluecrop, Coville and Jersey). Raw Weymouth berries showed slight anthocyanin leakage and also released some particulate matter during stirring, behavior not shown by the other samples and an indication that this sample was in poor condition. The sample apparently was not overripe, in fact having a low SS/A value (13.2) indicative of an early stage of ripeness (Woodruff et al., 1960).

Dewaxed blueberries behaved similarly, most samples releasing little or no anthocyanin (Fig. 1, Bluecrop). However, in some cases, minor leakage occurred, usually accompanied by turbidity. Absorbance vs time curves appeared to be linear (Fig. 1, Coville), sometimes preceded by a time interval during which leakage was minimal or absent (Fig. 1, Jersey). The latter behavior suggests the occurrence of a single event causing leakage such as the sudden puncturing or rupturing of the skin of one berry. Visible skin splitting was seen with dewaxed Weymouth berries, the more extensive anthocyanin leakage found with this sample being observed primarily in the vicinity of the skin ruptures. These results clearly show that removal of the epicuticular wax *per se* does not greatly affect anthocyanin leakage from raw berries, other barriers in the skin limiting the leakage rate. However, dewaxing may weaken the berry cuticle sufficiently to allow the skin to rupture, permitting some leakage from the exposed edges or undersurface of the torn skin.

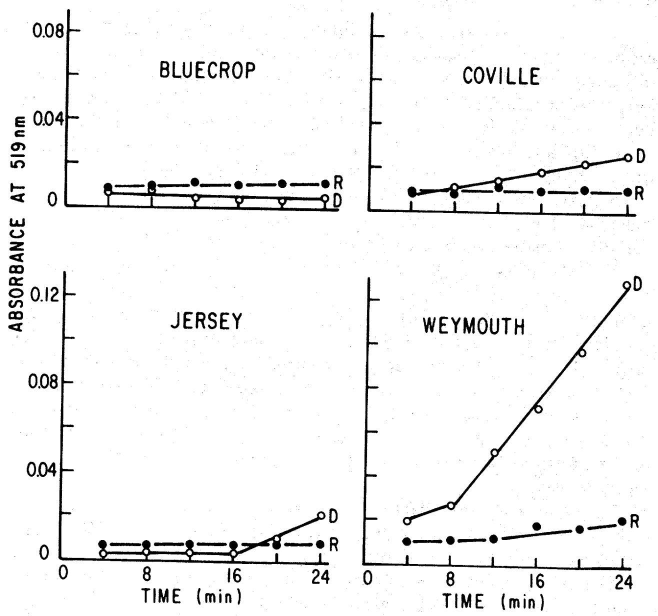

Fig. 1—Anthocyanin leakage from raw (R) and dewaxed (D) blueberries.

Anthocyanin leakage from stored blueberries

If blueberries are refrigerated for a prolonged period of time, some softening and decay will occur (Dekazos and Smit, 1976). When we evaluated sound, firm berries stored for as long as 5 wk (soft berries being removed from samples during sorting), we measured no anthocyanin leakage (Fig. 2), although the water in which the berries were stirred usually became turbid. However, with samples containing soft berries (not removed during sorting), significant anthocyanin leakage did occur. Leakage was accompanied by skin rupturing in as many as 10% of the berries being tested, a proportion similar to the percentage of soft berries in the sample (9% for Burlington and 15% for Elliott). Thus, anthocyanin leakage from stored blueberries depends on the extent to which individual berries undergo softening,

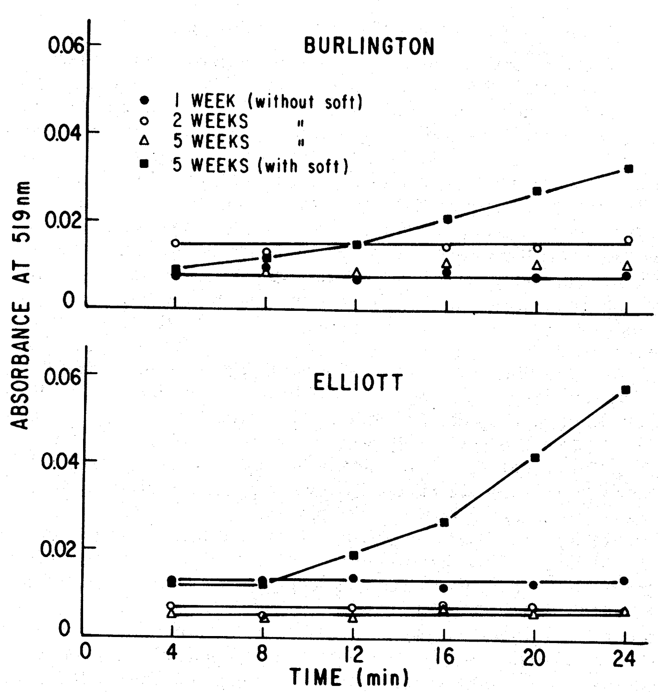

Fig. 2—Anthocyanin leakage from raw Burlington and Elliott blueberries after refrigerated storage.

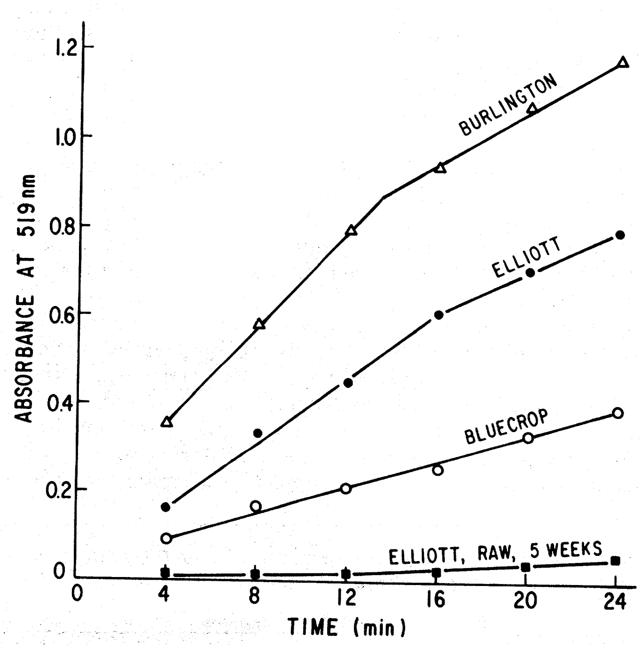

Fig. 3—Anthocyanin leakage from fresh, steam-cooked blueberries.

a consequence of "physiological breakdown" (Woodruff et al., 1960) and/or bruising (Ballinger et al., 1973) and not a generalized deterioration in the barrier properties of the skin affecting all berries.

Anthocyanin leakage from cooked fresh blueberries

Anthocyanin leakage from steam-cooked fresh blueberries greatly exceeded that from raw berries, even deteriorated berries stored 5 wk (Fig. 3). Absorbance vs time curves for the five cultivars compared usually were two-phase linear, the second slope being smaller than the first (see Burlington and Elliott). Occasional trials yielded linear or curvilinear leakage curves (see Bluecrop). Leakage rates (slopes 1 and 2, corresponding to each portion of the two-phase linear curves) varied greatly among replicates of the same sample and appeared to be related to the occurrence of berries having visible skin ruptures after 24 min stirring (Table 1). Correlation coefficients for slope 1 vs % ruptures were 0.94 and 0.78 for Elliott and Jersey, respectively). Leakage from skin ruptures in cooked berries entails the relatively unrestricted diffusion of pigments and other solutes from epidermal cells in the exocarp and the disorganized cell layers of the exposed surface. The greatly enhanced rate of leakage with cooked berries as compared to raw samples is evidence for the destruction by heat of the primary barrier to anthocyanin diffusion from the exocarp, the semipermeable plasma membranes of epidermal cells (Adams and Blundstone, 1971). Disruption of the epicuticular wax during cooking, indicated by the appearance of a wax film on the surface of the cooling water and water in which berries were stirred, as well as by the loss of bloom, also would remove a barrier to anthocyanin leakage from underlying tissues.

Cultivar differences in the rate of anthocyanin leakage (Table 2) were large, Bluecrop and Coville leaking less than Burlington and Elliott. If anthocyanin leakage were controlled by diffusion from the pigment-containing epidermal cells through the overlaying cuticle over the entire berry surface, one would expect leakage rates to be directly proportional to the berry surface area and anthocyanin content. Although these characteristics varied among the cultivars compared, correlations between them and leakage rates were marginally significant, surface area appearing to be less important than total anthocyanin (Table 3). The apparent inverse relationship between leakage rates and the SS/A ratio, an indicator of ripeness (Woodruff et al., 1960), may be fortuitous since the low ratio for Elliott reflects the high acidity that is characteristic of this cultivar rather than a lack of ripeness (Sapers et al., 1984a). The incidence of skin rupturing does not appear to explain differences in leakage rates between cultivars (see Table 2, Bluecrop vs Coville). However, the extent of skin rupturing, i.e., the dimensions of the rupture or exposed skin undersurface, which were not measured, may be more directly related to leakage than the incidence of skin rupturing. Both the incidence and severity of skin rupturing after cooking may be related to the thickness, tensile strength, and condition (damage due to weathering or handling) of the berry skin.

The occurrence of two-phase linear absorbance vs time curves suggests a change in the mechanism of leakage during the period of measurement, perhaps the depletion of one pigment source (i.e., a skin rupture) with subsequent diffusion from a less accessible pigment source (i.e., diffusion from epidermal cells through the cuticle) determining the leakage rate. The correlation between slope 2 and the total anthocyanin content and surface area is consistent with diffusion over the entire berry surface.

Both mechanisms, anthocyanin leakage through the cuticle and from skin ruptures, probably contributed to the coloration of cooking water, as observed previously (Sapers et al., 1984b). Such leakage may be desirable with canned products, for which a highly colored syrup is sought, but would be undesirable with baked products where diffusion of anthocyanins through the batter would produce an unattractive appearance. Cultivars such as Bluecrop and Coville would be superior to Burlington and Elliott for the latter application. Information on the causes of cultivar differences in the tendency of cooked blueberries to leak may be useful in selecting new cultivars for superior processability.

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Table 1—Anthocyanin leakage vs skin rupturing in steam-cooked blueberries

Cultivar	Trial	Berries with skin ruptures (%)	Leakage rate ($\times 10^{-3}$)[a] Slope 1	Slope 2
Elliott	1	27	71.0	—
	2	32	73.4	46.6
	3	38	64.6	—
	4	52	125.2	61.0
	5	62	158.0	87.2
Jersey	1	7	9.2	24.2
	2	21	44.0	—
	3	49	87.0	60.8
	4	50	89.2	45.6
	5	58	70.8	22.6

[a] Absorbance units per min per 100g berries

Table 3—Correlation between leakage rate and berry total anthocyanin content and surface area

Correlation	Correlation coefficient Slope 1	Slope 2
Slope vs surface area	0.400	0.560[b]
Slope vs TAcy[a]	0.824	0.878[b]
Slope vs surface area x Tacy	0.879[b]	0.923[b]

[a] Total anthocyanin content
[b] Significant at p = 0.05

Table 2—Anthocyanin leakage from steam-cooked blueberries

Cultivar	SS/A[a]	TAcy[b]	Total surface area (cm^2)	Berries with ruptures (%) Mean	Range	Leakage rate ($\times 10^{-3}$)[c] Slope 1 Trials	Mean	Range	Slope 2 Trials	Mean	Range
Bluecrop	21.0	66	166	15	12–18	3	54.3[e]	47.8– 62.6	4	25.0[f]	15.0–35.4
Burlington	13.5	152	230	44	35–53	4	95.6[d]	79.6–111.0	4	54.2[de]	38.0–64.4
Coville	22.0	143	186	58	38–77	4	66.4[e]	56.0– 77.2	5	30.5[f]	22.4–42.8
Elliott	11.9	233	207	42	27–62	5	98.4[d]	64.6–158.0	3	64.9[d]	46.6–87.2
Jersey	20.9	164	221	44	21–58	4	72.8[e]	44.0– 89.2	3	43.0[e]	22.6–60.8

[a] Soluble solids/titratable acidity
[b] Total anthocyanin = absorbance of ethanolic extract at 543 nm x dilution factor
[c] Absorbance units per min per 100g berries
[d–f] Means with different superscripts in same column are significantly different ($p < 0.05$)

CONCLUSIONS

Significant anthocyanin leakage from raw blueberries does not occur, except in stored berries that have undergone softening. The removal of the epicuticular wax has little effect on the extent of anthocyanin leakage from raw berries.

Cooking destroys the primary barrier to anthocyanin leakage, resulting in high leakage rates. Cultivar differences in anthocyanin leakage rates after cooking are due primarily to differences in the tendency of berry skins to rupture, and secondarily to berry anthocyanin content and surface area.

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