

Detection of Phenylalanine Ammonia-Lyase in the Skin of Blueberry and Cranberry Fruits

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ABSTRACT

Phenylalanine ammonia-lyase (PAL) was extracted from the skin of individual berries and assayed spectrophotometrically under conditions minimizing interference from co-extracted constituents. Analyses for total anthocyanin (TAcy), soluble solids (SS), and titratable acidity (A) were performed on the same berries. In blueberry fruits, PAL activity was present at all maturity levels examined. Cultivar differences in anthocyanin accumulation were unrelated to PAL activity. PAL activity in cranberry fruit was unrelated to harvest date, cultivar differences in anthocyanin accumulation, or values of TAcy and SS/A in individual berries. Activity was retained in cranberries during 4 wk of refrigerated storage.

INTRODUCTION

PREVIOUSLY, we have investigated the relationship between the fruit color and anthocyanin content of highbush blueberry (Sapers et al., 1984) and cranberry (Sapers et al., 1983, 1986a). The biosynthesis of anthocyanins in the skin of these fruits during ripening and post-harvest storage is of particular interest because of the possibility that coloration might be improved by stimulation of pigmentation through exposure to such environmental factors as ethylene and light (Cracker, 1971; Proctor and Creasy, 1971; Watanabe and Arakawa, 1983). The enzyme phenylalanine ammonia-lyase (PAL) plays a key role in anthocyanin biosynthesis, possibly controlling the extent of anthocyanin accumulation (Hahlbrock, 1981; Rhodes, 1983). PAL has been detected in various plant tissues (Hanson and Havir, 1981) including fruit parenchyma cell suspension cultures (Macheix et al., 1981; Sakamoto et al., 1980) but, to our knowledge, not in the skin of blueberry or cranberry fruit. Rivov et al. (1969) investigated the ethylene-controlled induction of PAL in citrus fruit peel, extracting the enzyme from an acetone powder of the peel tissue with borate buffer and partially purifying the enzyme by ammonium sulfate precipitation. Tan (1979) investigated the relationship between anthocyanin, PAL, and a PAL-inactivating system in apple skin, extracting the enzyme from a frozen powder, prepared by pulverizing the skin in liquid nitrogen, with borate buffer in the presence of polyvinylpyrrolidone (PVPP). The detection of PAL in pigmented fruit skins is likely to be complicated by the presence of phenolic compounds, quinones (generated from the former by polyphenol oxidase), and high concentrations of flavonoids which can complex or condense with proteins (Loomis, 1974) as well as interfere with the assay because of high background absorbance at 280 nm. Loomis (1974) has described the use of buffers, polymeric absorbents, antioxidants, and low temperature extraction to overcome these problems. Our objectives in the present study were to develop a PAL assay for the skins of individual small fruits that could be used in conjunction with other measurements to determine the relationship between PAL activity, the anthocyanin content, and fruit ripeness, and to apply this procedure to ripening blueberry and cranberry fruits.

MATERIALS & METHODS

Experimental materials

Blueberry and cranberry samples, harvested at the Rutgers University Blueberry and Cranberry Research Center (Chatsworth, NJ) in 1984, were stored at -29°C until assayed. Blueberry fruits of several cultivars, representing different stages of color development, were harvested at Chatsworth in July, 1985, and stored briefly at 3°C until assayed. Cranberry fruits that had been tagged at the pink-white stage of color development were harvested at intervals and then assayed immediately and again after 4 weeks storage at 3°C . Samples of other fresh fruits were obtained at local supermarkets. PAL (EC 4.3.1.5; purified from *Rhodotorula glutinis*) was obtained from P.L. Biochemicals, Inc. (Milwaukee, WI) or from Sigma Chemical Co. (St. Louis, MO) and stored at -18°C . PVPP (Sigma Chemical Co.) was purified by the method of Loomis (1974). Amberlite XAD-7 polymeric adsorbent (Rohm and Haas Company, Philadelphia, PA) was purified by a water-washing procedure, as recommended by the manufacturer (Anon., 1969).

Extraction and purification procedure

The skin of individual weighed fruits (fresh or frozen) was completely removed with a razor blade and tweezers and immediately frozen, together with the peeled fruit, in liquid nitrogen. The frozen skin fragments were ground in liquid N_2 to a fine powder with a precooled mortar and pestle. To extract PAL and the anthocyanins, the frozen powder was ground with 5 mL 20 mM 2-mercaptoethanol in 0.1M sodium borate buffer, pH 8.8, and 0.4g purified PVPP. The resulting suspension was then quantitatively transferred to a 1 cm diam PVPP column, prepared with 2.5g moist, purified resin (1g dry PVPP = 3.5g moist PVPP), and held at 4°C . PAL was recovered from the column by elution with 15 mL distilled H_2O . Anthocyanins were recovered from the PVPP column following PAL recovery by elution with 20–60 mL 95% ethanol:1.5M HCl (85:15).

PAL assay

Aliquots of dilute PAL solutions, fruit skin extracts, and column eluates not exceeding 5 mL in volume were diluted to 10 mL with 0.1M sodium borate buffer, pH 8.8, in 16×125 mm screw cap tubes. Five milliliters 0.06M L-phenylalanine were added to each tube at zero time. A reagent blank containing borate buffer in place of the PAL solution and an enzyme blank containing distilled H_2O in place of L-phenylalanine solution also were prepared. The tubes were capped, gently mixed by inversion, and incubated for 90 min in a 37°C water bath. The absorbance of the reaction mixtures and enzyme blank was determined against the reagent blank at 280 nm at 10 min intervals with a Perkin-Elmer Model 552 UV-visible spectrophotometer, equipped with a Perkin-Elmer Super Sipper having a temperature-controlled micro-flow cell set to 37°C . PAL activity was calculated from the slopes of absorbance vs time curves, obtained by linear regression, a unit of activity being defined as the production of 1 μmole cinnamate per min at 30°C (Havir and Hanson, 1970). A molecular extinction coefficient for cinnamate of 16,100, which is equivalent to 0.932 μmoles per absorbance unit under the conditions of the assay, was determined experimentally. Since enzyme blanks underwent little or no change in absorbance during the assay, no enzyme blank correction was required. A temperature correction factor of 0.598, corresponding to an Arrhenius activation energy of 13.7 kcal/mole, was used to convert the activity values from 37° to 30°C , as described by Havir and Hanson (1970). Thus, the activity was calculated as:

$$\text{Activity (units)} = \text{Slope} \times \text{Dilution factor} \times 0.557$$

where 0.557 is the product of 0.932 and 0.598.

Determination of total and individual anthocyanins

The total anthocyanin content of fruits assayed for PAL was determined by spectrophotometric analysis of the alcoholic column eluates at 543 nm for blueberry and at 535 nm for cranberry. Concentrations were expressed as mg anthocyanin per 100 ml for cranberry, based on the extinction coefficients determined by Fuleki and Francis (1968a); concentrations were expressed as absorbance units (A.U.) per 100 mL for blueberry, since extinction coefficients were not available. Because the anthocyanins were derived entirely from the skin, the anthocyanin contents of these berries was expressed as mg or A.U. per cm² of surface area, calculated from the quantity of anthocyanin eluted from the column and an estimate of the surface area, obtained from the berry weight, W, based on the assumption that the berries were spherical and had a density of 1.04 (Sapers and Phillips, 1985).

$$\text{Surface area (cm}^2\text{)} = 4.71 W^{2/3}$$

The proportions of individual anthocyanins in cranberry juice and alcoholic eluates was determined by HPLC, as described by Sapers et al. (1986b).

Determination of fruit soluble solids-acidity ratio (SS/A)

Each frozen peeled berry was weighed, shattered into fragments with a mortar and pestle, and transferred to a stainless steel micro-blender jar with an equal weight of distilled H₂O. After about 1 min to allow for partial thawing, the berry was homogenized at high speed for 2 min. A drop of homogenate was used to obtain the soluble solids content by refractometry with a B & L Abbe -3L Refractometer (Bausch & Lomb, Inc., Rochester, NY). Weighed quantities of homogenate were diluted with 50 mL distilled H₂O and titrated with 0.1M NaOH to a pH 8.1 endpoint. Values of SS/A were calculated as the ratio of the percent soluble solids (corrected to 20°C) to the titratable acidity (expressed as percent citric acid).

Evaluation of PVPP column technique

The PVPP column technique was evaluated with buffered cranberry juice containing added PAL rather than with extracts of individual berry skins which would be highly variable in composition and sufficient only for a few trials. The juice was pressed from thawed cranberry samples (Sapers et al., 1983), adjusted to pH 4, 5 or 7 with borate buffer, and spiked with varying amounts of PAL (in borate buffer), added after pH adjustment. Juice samples (which contained no PAL activity), were analyzed spectrophotometrically for total anthocyanin by the pH differential method of Fuleki and Francis (1968b). PAL assays were performed daily on enzyme solutions used in each experiment. PVPP columns equilibrated at 20° or 4°C, were charged with varying amounts of juice at different pH values and added PAL levels. PAL was eluted, and the percent recovery of enzyme activity and percent removal of substances absorbing at 280 nm were calculated. The anthocyanins were then eluted, and the percent recovery determined. Additional recovery experiments more closely resembling berry skin assays were performed with simulated skin extracts comprising 0.5 mL cranberry juice, diluted with 0.6, 1.0, 1.7 or 4.0 mL borate buffer containing 20 mM 2-mercaptoethanol and sufficient water to bring the final volume to 5 mL, giving pH values of 4.2, 5.1, 7.4, or 8.0, respectively. Each simulated extract was combined with 0.4g PVPP and 0.005 units of PAL and then added to a 2.5g PVPP column, equilibrated at 4°C, for PAL and anthocyanin elution.

RESULTS & DISCUSSION

Preliminary experiments with purified PAL

Assays performed on dilutions of *Rhodotorula glutinis* PAL in pH 8.8 borate buffer, to determine the lower limits of PAL detection, gave a constant activity per milliliter of enzyme concentrate for slopes between 0.5×10^{-3} and 14.5×10^{-3} absorbance units per min. A highly variable response was observed with more dilute enzyme solutions, i.e., those giving slope values smaller than 0.5×10^{-3} absorbance units/min, which is equivalent to a PAL concentration of about 2×10^{-5} activity units/mL. Ideally, fruit skin samples to be assayed for PAL should be large enough to contain 3×10^{-4} activity units for the assay to be in the useable slope range, assuming that the fruit skin PAL is similar in behavior to PAL from *R. glutinis*.

Attempts to demonstrate PAL activity in extracts of cranberry, blueberry and blackberry fruits prepared by blending the berries and borate buffer for 2 min at high speed in a stainless steel semi-micro blending jar on a Waring base, adding 5% Celite Analytical Filter Aid to the homogenate and then filtering through Whatman No. 5 paper under suction, were unsuccessful, even when the berries were "spiked" with PAL prior to blending. Blending PAL in buffer (no added berries) without any further treatment was sufficient to cause the loss of all PAL activity. PAL activity also was completely lost when 0.5–5.0% Celite was added to borate buffer solutions of the purified enzyme or to spiked cranberry homogenates diluted with buffer and then removed by filtration through Whatman No. 5 paper under suction prior to the assay. About half of the added PAL activity was lost when solutions of the enzyme in borate buffer or in previously clarified buffer extracts of cranberry, blueberry or blackberry fruits were filtered through Whatman No. 5 paper without added Celite. These berry extracts contained no endogenous PAL activity (as a consequence of blending and Celite addition) and, when spiked with PAL, were equivalent in enzyme activity to spiked borate buffer.

Finally, PAL activity was completely lost when cranberry juice was spiked prior to dilution with borate buffer. The loss in activity could be avoided by buffering the highly acidic juice (pH 2.5) to pH 5 (juice:buffer = 1:2) before the addition of PAL.

Thus, extraction procedures that entail high speed blending, the use of Celite, filtration, or prolonged exposure of the extracted PAL to a low pH due to co-extracted fruit acids should be avoided.

PVPP column for sample clean-up

To perform assays for PAL in intensely colored fruit skin extracts, interfering flavonoids and other phenolic compounds must first be removed. We investigated the use of PVPP columns to remove these compounds from cranberry juice spiked with PAL and also examined the recovery of adsorbed PAL and anthocyanins from the spent PVPP for subsequent quantitation (Table 1).

All treatments reduced the absorbance of extracts at 280 nm by 70–90%, presumably by the adsorption of flavonoids (including the anthocyanins), polyphenols, and other aromatic compounds on the column. The pH had a marked effect on PAL recovery, the enzyme being inactivated at pH 4; anthocyanin recovery was greater at the lower pH values than at pH 7 (Expt. 1). PAL recovery increased and anthocyanin recovery decreased as the quantity of cranberry juice loaded on the column decreased (Expt. 2). We estimate from the mean berry weights and anthocyanin contents of representative cranberry cultivars that the 1 mL juice level was equivalent in pigment content to the skin of 1 large berry (Sapers et al., 1986c). Column size (Expt. 3) had little if any effect on PAL or anthocyanin recovery. PAL recovery was improved when the column treatment was carried out at 4°C instead of at room temperature (Expt. 4). PVPP columns were compared with columns containing Amberlite XAD-7, alone or mixed with PVPP. Higher PAL recoveries were obtained with PVPP columns than with the mixed resins (Expt. 5) or XAD-7 alone (data not shown). Consequently, PVPP columns were used for extract clean-up in all subsequent PAL assays. PAL recovery decreased when the level of enzyme added to the column was reduced from 0.008 to 0.002 units (Expt. 6). This may be due in part to the variability of the assay at low PAL concentrations as well as to losses in activity during extraction and clean-up on the PVPP column.

Under conditions more closely simulating the assay of berry skins (Expt. 7), the PVPP column reduced the absorbance of simulated extracts at 280 nm by 70–78% and gave PAL recoveries of 78–87% as well as anthocyanin recoveries of 88–

Table 1—Performance of PVPP column in clean-up of cranberry juice for PAL assay.

Expt.	Treatment				Column temp (°C)	Performance		
	Column size (g PVPP) ^a	Added PAL (units)	Added cranberry juice (mL)	pH		A280 removed (%)	PAL recovery (%)	Anthocyanin recovery (%)
1	1.8	0.011	4	4	20	78	0	95
	1.8	0.011	4	5	20	89	50	91
	1.8	0.011	4	7	20	85	65	31
2	1.8	0.011	2	5	20	85	68	78
	1.8	0.012	1	5	20	85	88	36
3	1.8	0.010	1	5	20	91	74	56
	2.5	0.010	1	5	20	91	76	52
4	1.5 ^b	0.009	1	5	20	90	57	—
	1.5 ^b	0.009	1	5	4	90	69	—
5	1.5 ^b	0.006	1	5	4	92	59	—
	1.5	0.006	1	5	4	89	77	—
	2.5	0.006	1	5	4	90	87	—
6	2.5	0.008	1	5	4	89	109	110
	2.5	0.004	1	5	4	92	70	113
	2.5	0.002	1	5	4	93	24	111
7 ^c	2.5	0.005	0.5	4	4	82 ± 3	35 ± 15	91 ± 8
	2.5	0.005	0.5	5	4	78 ± 2	78 ± 10	98 ± 8
	2.5	0.005	0.5	7	4	76 ± 5	87 ± 9	95 ± 8
	2.5	0.005	0.5	8	4	70 ± 3	83 ± 4	88 ± 4

^a Moist, purified PVPP packed in 1 cm diam column.

^b 1:500 dilution of PAL standard.

^c 1.5g PVPP + 0.75g XAD-7.

^d Determined in triplicate; mean values ± standard deviation.

Table 2—Recovery of individual anthocyanins from PVPP column used in PAL assay of cranberries

Trial	Peak no. ^b	Peak area (%) ^a	
		Juice added ^c to column	Alcoholic eluate ^d from column
1	1	27.4	23.5
	3	13.0	12.1
	4	39.5	41.6
	5	4.2	5.2
	6	12.2	13.6
	2	1	27.2
3		13.0	13.1
4		39.6	38.8
5		4.1	4.8
6		12.3	12.7

^a Mean of duplicate determinations for identical columns (1 and 2) prepared on successive days.

^b Peaks 1, 3, 4 and 6 tentatively identified as cyanidin-3-galactoside, cyanidin-3-arabinoside, peonidin-3-galactoside, and peonidin-3-arabinoside, respectively. Peak 5 not identified. (Sapers and Hargrave, 1987).

^c 1 mL Ben Lear cranberry juice, pH 5, added to 2.5g PVPP column at 4°C.

^d Eluted with 40 mL 95% ethanol:1.5M HCl (85:15).

Table 3—Effect of PVPP and 2-mercaptoethanol addition during extraction on the level of PAL detected in the skin of Santa Rosa plum

Expt.	Treatment	PAL activity in skin (units/g × 10 ⁻³)
I	Control	0.87
	10 mM 2-mercaptoethanol	3.50
	20 mM 2-mercaptoethanol	6.72
	40 mM 2-mercaptoethanol	3.76
II	Control	0.97
	0.1g PVPP	1.08
	20 mM 2-mercaptoethanol	1.84
	0.1g PVPP + 20 mM 2-mercaptoethanol	3.64
III	Control	1.58
	0.4g PVPP	1.18
	20 mM 2-mercaptoethanol	2.56
	0.4g PVPP + 20 mM 2-mercaptoethanol	2.85

98% at pH values of 5–8. As expected, PAL recovery was much lower at pH 4.

To determine whether the column treatment had any effect on individual anthocyanins adsorbed from berry skin extracts,

we compared the proportions of individual anthocyanins, determined by HPLC, in cranberry juice and in alcoholic eluates from a PVPP column loaded with 1 mL of the same juice (Table 2). The proportions of individual anthocyanins were very similar in the original juice samples and column eluates. Consequently, the anthocyanin composition of a berry being assayed for PAL activity could be determined by means of HPLC analysis of the alcoholic eluate.

Detection of PAL in fruit skin

Initial efforts to demonstrate PAL activity in fruit skins ground in liquid N₂, extracted with cold borate buffer, and assayed following PVPP column pretreatment, were successful with Santa Rosa plum and sweet cherry but not cranberry (frozen) or apple. When the borate buffer used to extract frozen cranberry skin powder was spiked with 0.0027 units PAL, about 50% of the added activity could be recovered in the column eluate following PVPP clean-up.

To obtain further improvements in PAL recovery from fruit skin, 2-mercaptoethanol and PVPP were added to the borate buffer used to extract the frozen, powdered skin (Table 3). These experiments were performed with skin from Santa Rosa plums instead of blueberries or cranberries (which have only about 5-10% of the surface area of a plum) so that sufficient material of similar composition would be available for the evaluation of multilevel treatments. The recovered PAL activity was greatest when the buffer contained 20 mM 2-mercaptoethanol (Expt. I). PVPP was ineffective by itself but consistently increased the level of detected PAL activity when used in combination with 2-mercaptoethanol (Expts. II and III). The combination of 20 mM 2-mercaptoethanol and 0.4g PVPP was employed in subsequent assays for PAL in fruit skin, the amount of PVPP conforming to the use level recommended by Loomis (1968). Extracts obtained with this combination had pH values between 7.7 and 8.6, depending on the acidity and mass of the berry skins. With the modified extraction procedure, we were able to detect PAL activity in the skins of fresh blueberries (2.3 × 10⁻³ units/g) and frozen cranberries (0–3.5 × 10⁻³ units/g) but not in nectarine or peach skins. The recovery of PAL from samples of blueberry and cranberry skin spiked with 0.0040 enzyme units exceeded 80% (with correction for endogenous PAL).

Table 4—PAL activity in the skins of individual blueberry fruits of different coloration

Cultivar	Berry color	No. berries assayed	Mean ± Standard deviation		
			PAL activity (units/cm ² × 10 ⁻⁴) ^a	Total anthocyanin (A.U./cm ²) ^{a,b}	SS/A
Bluecrop	Pink-green	2	0	0.3 ^c	0.7 ^c
	Red-purple	3	1.1 ± 0.1	1.5 ^c	4.5 ^c
	Purple-blue	3	1.9 ± 0.5	3.0 ^c	6.5 ^c
	Blue	2	0.8 ± 0.1	18.3 ^c	10.0 ^c
	Black	4	1.6 ± 0.8	12.9 ^c	42.6 ^c
Coville	Pink-green	2	0.5 ± 0.6	0.4 ± 0.0	2.4 ± 0.2
	Red-purple	2	1.5 ± 0.0	2.8 ± 0.4	5.0 ± 1.1
	Purple-blue	2	0.8 ± 0.1	6.2 ± 2.3	5.4 ± 0.4
	Blue	2	0.6 ± 0.2	27.0 ± 12.3	13.6 ± 1.2
	Black	2	0.6 ± 0.2	25.9 ± 9.8	20.6 ± 3.7

^a Calculated from estimated surface area of each berry.

^b A.U. (absorbance unit) = absorbance at 543 nm × volume × dilution factor.

^c Single berry only.

Table 5—PAL activity vs total anthocyanin and SS/A in ripening cranberry fruit after harvest and following 4 wk storage at 3°C

Cultivar	Days after tagging ^b	Mean ± standard deviation ^a					
		After harvest			After storage		
		PAL (units/cm ² × 10 ⁻⁴) ^c	Total anthocyanin (mg/cm ²) ^c	SS/A	PAL (units/cm ² × 10 ⁻⁴) ^c	Total anthocyanin (mg/cm ²) ^c	SS/A
Franklin	0	1.5 ± 0.7	0.009 ± 0.006	3.6 ± 0.7	2.8 ± 0.5	0.044 ± 0.045	3.1 ± 0.5
	7	3.1 ± 1.6	0.089 ± 0.066	4.2 ± 0.9	2.1 ± 0.6	0.046 ± 0.022	3.2 ± 0.3
	15	1.6 ± 0.6	0.044 ± 0.030	4.1 ± 1.7	2.4 ± 0.6	0.053 ± 0.031	3.6 ± 0.6
	21	2.2 ± 0.3	0.057 ± 0.035	3.6 ± 0.1	3.0 ± 0.9	0.089 ± 0.030	4.2 ± 0.6
Howes	0	3.4 ± 0.5	0.006 ± 0.001	2.9 ± 0.4	2.3 ± 1.3	0.014 ± 0.012	2.9 ± 0.1
	7	3.8 ± 1.2	0.030 ± 0.019	3.9 ± 0.2	2.6 ± 0.4	0.023 ± 0.016	3.6 ± 0.3
	15	2.8 ± 0.8	0.054 ± 0.033	3.7 ± 0.6	3.2 ± 1.1	0.079 ± 0.019	3.9 ± 0.7
	21	2.5 ± 0.4	0.071 ± 0.064	4.5 ± 0.8	2.4 ± 0.4	0.087 ± 0.046	3.5 ± 0.4

^a Mean of four berries per treatment.

^b Tagged at pink-white stage of color development.

^c Calculated from estimated surface area of each berry.

Quantitative studies with blueberry and cranberry

To determine whether PAL activity in blueberry was related to anthocyanin content or ripeness, we carried out a quantitative study of blueberry fruits representing 2 cultivars, harvested at different stages of color development (Table 4). Our data indicated somewhat variable PAL activity in fruits of different maturity, as indicated by coloration, total anthocyanin, and SS/A values. Correlations between PAL activity and total anthocyanin or SS/A were not significant for either cultivar. The presence of PAL in fully ripe and over-ripe blueberries is probably of marginal importance with respect to fruit color, even if anthocyanin biosynthesis continues in the skin, since the color of such fruit is greatly influenced by the state of their epicuticular wax, i.e., the presence of a rodlet wax structure which produces the glaucous effect responsible for the typical light blue color of blueberries (Albrigo et al., 1980).

Although the presence of PAL in the skin of ripening blueberries may be related in some way to their capacity to accumulate anthocyanin, there was no relationship between PAL activity in Coville berries which are high in total anthocyanin content (150 A.U./g in fully ripe berries) and in Bluecrop which are low (63 A.U./g). It would be of interest to extend the methodology described herein to other enzymes involved in anthocyanin biosynthesis as well as to potential substrates and inhibitors so that the basis for genetic differences in total anthocyanin accumulation and in the pattern of individual anthocyanins observed in previous studies (Sapers et al., 1984) could be elucidated.

The level of PAL activity found in frozen cranberry samples (data not shown) varied greatly between berries but did not appear to be related to berry coloration or cultivar. Due to the possibility that these results may have been influenced by the frozen storage of the fruit (8 months at -29°C), we repeated the study with fresh cranberries that were tagged in the pink-white stage of color development and then harvested at 1 wk

intervals over 3 wk. Analytical data obtained for two cultivars at the time of harvest and after 4 wk at 3°C (Table 5) indicated no relationship between PAL activity and harvest date or post-harvest storage. Similarly, we observed no correlations between the PAL activities of individual berries and their total anthocyanin contents ($r = 0.46$ and -0.16 for Franklin and Howes cranberries, respectively) or SS/A values ($r = -0.19$ and -0.04 for Franklin and Howes respectively). Values of the latter parameter are indicative of cranberry fruit ripeness (Sapers et al., 1986a). Our data did not show the extent of post-harvest color development since we intentionally selected berries representing a range of colorations at each sampling time to permit the comparison of PAL activity with total anthocyanin content. However, the presence of PAL activity in fruits of different ripeness and anthocyanin content after 4 weeks of post-harvest storage is consistent with the well known ability of cranberries to increase in pigment content during storage (Zukerman et al., 1966).

In a previous study (Sapers et al., 1986c), we reported large differences in the capacity of Franklin and Howes cranberries to accumulate anthocyanin, fully colored fruits of the former cultivar containing twice as much pigment as the latter. This characteristic does not appear to be related to PAL activity in the fruit skin. The extent to which PAL or other enzymes, substrates, or inhibitors limit anthocyanin accumulation in ripening cranberries and during post-harvest storage requires further investigation. Such information may shed light on genetic differences in anthocyanin accumulation and suggest new approaches to the enhancement of cranberry fruit color.

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