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Characterization of Anthocyanin-Containing Colorants and Fruit Juices by HPLC/Photodiode Array Detection†

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The anthocyanin pigment profiles of commercial black currant, blackberry, black raspberry, elderberry, cherry, plum, grape, bilberry, and red cabbage products were characterized by high-performance liquid chromatography (HPLC)/photodiode array detection. Removal of acylated anthocyanins by alkaline hydrolysis and selective removal of anthocyanins on a reversed-phase cartridge with borate buffer were auxiliary techniques that proved helpful in making peak assignments. Both the retention properties on reversed-phase HPLC and the spectral properties by photodiode array detection were used to characterize the anthocyanins. Other properties including tinctoral strength, total anthocyanin concentration, browning, titratable acidity, and Hunter tristimulus values were also determined for these colorants.

The recent banning of Red No. 2 in the United States along with the questionable status of Red No. 40 has led to the increased use of natural red pigments as coloring agents (Wallin and Smith, 1977). The anthocyanins are one such class of natural red pigments that have found use as a suitable alternative for synthetic colorants in many applications.

The anthocyanins are widely distributed in nature, occurring in most higher plants. They are found in all parts of the plant but are most obvious in fruits and flowers (Brouillard, 1982). Pigment extracts are commercially available, grape skin extract (GSE) being the most common. In the United States only two sources of anthocyanin extracts are allowed to be used in foods. GSE is restricted for use in coloring beverages while grape color extract (GCE), an extract of concord grapes, can be used to color nonbeverage foodstuffs (Office of the Federal Register, 1986). In the European Economic Community (EEC) countries, anthocyanin extracts from food sources are generally allowed (for specific information this class of colorants is listed under EEC E163).

In addition to extracts, the concentrated juice of red fruits such as cranberries, raspberries, elderberries, etc., can also be used in food products that are compatible with the acidity and flavor of the fruit juice concentrate involved (Riboh, 1977). In the United States and for most EEC countries, fruit juice and concentrates can be used without restriction.

The demand for anthocyanin-containing colorants is increasing. Fruit juice concentrates have become an important ingredient in the manufacture of many foods and beverages. Production of fruit juice concentrates has become highly competitive in both domestic and foreign markets. Analytical methodology able to identify the anthocyanins and to determine their source are needed for quality control and for determination of the authenticity of fruit juice concentrates and color extracts as well as for regulatory activities (Wrolstad et al., 1981).

Chemical analysis of plant constituents (chemotaxonomy) is an excellent objective method for identification and classification of plants. The anthocyanin composition of many fruits is quite distinctive (Wrolstad et al., 1981), and analysis of anthocyanins has been successfully used to detect adulteration of Concord grape juice with *vinifera* or hybrid varieties (Mattick et al., 1967; Fitelson, 1967), adulteration of blackberry and cherry juice with elderberry or grape skin extract (Fitelson, 1968), and adulteration of cranberry juice cocktail with grape skin extract (Hale et al., 1986; Hong and Wrolstad, 1986).

The objectives of this work are 2-fold: First, the general coloring properties of several commercially available anthocyanin colorants and juice concentrates are compared. This information is useful in product development and in quality assurance. The data presented may be helpful in selecting a colorant with the appropriate hue and/or tinctoral strength. The second objective is to use HPLC coupled with photodiode array detection

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to make peak assignments and characterize anthocyanin profiles. This information is useful from a regulatory standpoint such as identification of an added food colorant or detection of adulteration in anthocyanin-containing products.

MATERIALS AND METHODS

Samples. *Colorants.* San Ei San Red ELF (elderberry colorant) and red cabbage colorants: San Red RCF, San Red RC liquid, and San Red RC powder were supplied by G. Shimizu and Co., Ltd. (Osaka, Japan); Obi-Pektin black currant powder (200), Obi-Pektin elderberry (200), Spreda Enocianine powder, Type 501 (designated Spreda 501), Obi-Pektin bilberry powder (250), Obi-Pektin Morello cherry powder (348), Obi-Pektin Morello cherry powder (240), and Obi-Pektin Morello cherry powder (240 D) were supplied by Spreda (Burgdoff, Switzerland). Welch's grape colorant, Type 250, was supplied by Welch Foods (Westfield, NY). An additional sample of Spreda grape skin extract 501 (designated Spreda GSE) was supplied by General Foods, Inc. (New York).

Juice Concentrates. Plum, black raspberry, and blackberry juice concentrates were supplied by Kerr Concentrates (Salem, OR).

Red FD&C 40 was obtained from International Flavors and Fragrances (New York, NY).

Red raspberry juice pressed in our laboratory was available from a previous study (Spanos and Wrolstad, 1987).

Spectral and Acidity Analyses. Wavelength maxima, anthocyanin concentration as cyanidin 3-glucoside (ϵ = 29 600), degradation index, color density, polymeric color, percent tannins, and browning index were measured by the procedure described by Wrolstad (1976).

Tinctoral strength was expressed as the absorbance at the visible wavelength maximum of a 1% solution of the pigment in the appropriate solvent, either distilled water or pH 1 buffer, as described by Wrolstad (1976). A 1% stock solution of the pigment in the appropriate solvent was made and diluted such that the absorbance at the visible maximum was less than 1. Absorbance readings were taken, and the tinctoral strength was calculated by multiplication with the dilution factor.

Hunter tristimulus values (Hunter DP-25P-2, Hunter Instruments, Reston, VA) were determined both on 1% solutions (in distilled water) of the colorants and at constant absorbance where the pigment solutions (in pH 3.5 buffer) were diluted such that the absorbance at the visible wavelength maximum was 1. The procedure is described in detail by Sapers et al. (1981). All Hunter colorimeter measurements were made with 1-cm (i.d.) cells with the instrument in the transmittance mode (specular component included).

Titratable acidity was determined by the glass electrode method, AOAC 22.059 (AOAC, 1984).

Chromatographic Methods. HPLC methodology for analysis of anthocyanidins and anthocyanins is described by Hong and Wrolstad (1988).

Photodiode array measurements of spectral properties for the individual peaks were determined at the apex of the peak except in the case of closely eluting peaks. For closely eluting peaks, spectral measurement were determined at the front of the peak for the first peak and at the rear of the peak for the second peak.

Alkaline hydrolysis data are reported as follows. Chromatograms of both the untreated sample and alkaline hydrolyzed samples were compared. Peaks remaining in the alkaline hydrolyzed sample were reported as not hydrolyzed while peaks present in the untreated chromatogram and not in the alkaline hydrolyzed sample was reported as being hydrolyzed.

Nonacylated anthocyanins containing adjacent hydroxy groups are selectively removed from a reversed-phase minicolumn by alkaline borate buffer. The details of this procedure have been previously described (Hong and Wrolstad, 1990). The data for these analyses are reported as follows: Chromatograms of an untreated sample and the retained anthocyanins from the borate procedure were compared. A peak height ratio of the peak in question to that of a known delphinidin or cyanidin glycoside was determined. If the relative height of the peak in question was found to increase by at least 2 times in the borate treatment chromatogram (when compared to the untreated sample), then the peak in question was considered to be retained; otherwise, the peak was reported as being not retained. Peaks not retained are likely to be nonacylated cyanidin or delphinidin glycosides.

RESULTS AND DISCUSSION

Sample Information. A variety of different anthocyanin-containing colorants were examined, including fruit juice concentrates which are readily available and may be used without restriction in the United States. The Obi-Pektin samples all come as powders. The manufacturer has stated the following specifications for these products: Morello cherry 240-D, 40% dry fruit material, 60% maltodextrin added, vacuum-dried; Morello cherry 240, 40% dry fruit material, 60% sucrose added, vacuum-dried; Morello cherry 348, 48% dry fruit material, 12% sucrose, 37% starch, 3% citric acid, drum-dried; bilberry 250, 50% dry fruit material, 50% sucrose added, vacuum-dried; blackcurrant 200, 100% dry fruit powder, vacuum-dried; elderberry 200, not specified. All of the above products are essentially dried fruit juices. From a regulatory standpoint, these products may be legally used as food ingredients in the United States.

The remaining samples are extracts of anthocyanins from natural sources. Enocianin powder A (Spreda GSE Type 501) is a spray-dried product specified by the manufacturer to contain 40–70% dry fruit material with 30–60% maltodextrin added. San Red RC, RC powder, and RCF are anthocyanin extracts derived from red cabbage (*Brassica oleracia* var. rubla). The manufacturer states the following specifications for their colorants: San Red RC, 15% propylene glycol, 1% citric acid; San Red RC powder, 36% red cabbage color, 2% citric acid, 62% starch syrup powder; San Red RCF, 20% ethyl alcohol, 2% citric acid. San Red ELF is an extract of elderberry anthocyanins. The extract is specified to contain 20% ethyl alcohol and 5% citric acid. The Welch's grape color extract is declared to be an extract prepared from citric acid extraction of Concord grapes. Of the colorants in this group, only the grape samples are permitted to be used in the United States. As previously discussed, enocianin A is allowed to be used only in beverages while Welch's grape color extract is allowed to be used only in nonbeverage applications (Office of the Federal Register, 1986).

General Colorant Properties. Listed in Table I are the general properties of 13 colorants and 2 juice concentrates (blackberry and plum). The concentration of monomeric anthocyanins was measured by both the pH differential method and by the single-pH method (Wrolstad, 1976). All values are expressed as cyanidin 3-glucoside even if the sample did not contain major quantities of this pigment, allowing for easy comparison in total anthocyanin concentrations between different commodities. Both of the Spreda GSE samples, the San Ei elderberry colorant, and all of the cabbage colorants were high in total anthocyanin. This is not unexpected as they are all concentrated pigment extracts. In addition to the contribution of color by the monomeric anthocyanins, there can be contribution of color from polymerized anthocyanins and brown pigments arising from enzymic and nonenzymic browning (Wrolstad, 1976). A useful index for relative amount of polymeric color in these pigments can be determined from their resistance to bleaching by bisulfite. This is expressed as percent polymeric color. In general, the percent polymeric color values in all of the samples analyzed were close to 50%, indicating that

Table I. Comparison of General Colorant Properties for Commercial Colorants and Juice Concentrates

	Red FD&C 40	San-Ei elder-berry	Obi-Pektin elder-berry	Obi-Pektin black currant	black-berry conc	Spreda GSE	Speda GSE 501	Welch's grape color extract	Obi-Pektin Morello 348	Obi-Pektin Morello 240	Obi-Pektin Morello 240D	Obi-Pektin bil-berry	plum conc	San Red RCF	San Red RC liq	San Red RC powder
λ_{max}, nm (pH 1 buffer)	496	518	514	517	513	523	520	519	516	15	516	521	512	528	531	529
pH of 1% soln		3.0	4.4	3.2	3.3	3.0	3.4	3.2	3.4	3.6	3.6	3.3	3.5	3.2	3.2	3.3
titratable acidity[a]		5.5	6.3	20.4	10.6	11.8	7.1	4.7	7.1	3.8	4.4	4	5.6	2.7	2.9	3.8
monomeric anthocyanin concn,[b] mg/L for 1% soln		88.3	29.5	27.7	37.7	126.6	226.4	48.7	5.1	2.9	4.8	18.3	3.7	100.7	95.5	244.9
anthocyanin concn,[c] mg/L for 1% soln		137.6	44.2	38.4	48.4	195.0	267.2	55.8	7.9	5.2	6.9	26.0	5.3	136.7	158.6	366.9
degradation index		1.6	1.5	1.4	1.3	1.5	1.2	1.1	1.6	1.8	1.4	1.4	1.4	1.4	1.7	1.5
color density		5.6	6.5	2.1	1.6	8.9	5	4.6	0.4	0.3	0.3	1	0.3	3.3	5.6	9
polymeric color		2.5	3.8	0.9	0.7	3.2	1.6	0.8	0.2	0.2	0.1	0.4	0.1	0.4	0.7	0.8
% tannins		45	59	43	44	35	32	16	45	50	35	41	48	13	12	9
browning index		1.3	3.1	0.6	0.5	1.5	0.8	0.5	0.1	0.1	0.1	0.2	0.1	0.3	0.5	0.6
tinctoral strength (abs of 1% soln)		3.3	2.7	1.0	0.8	5.8	3.3	0.7	0.2	0.2	0.2	0.6	0.1	2.4	4.2	6.8
tinctoral strength at pH 1 (abs of 1% soln)	409.2	9.2	2.9	2.6	3.2	13.0	18.7	2.1	0.5	0.4	0.5	1.7	0.4	9.1	10.6	24.4
Hunter parameters (1% soln)																
L	x[f]	18	29	45	40	x	x	31	79	83	84	51	87	28	21	18
a	x	40	26	46	45	x	x	51	18	10	13	44	9	60	48	41
b	x	12	15	22	14	x	x	10	8	8	4	7	6	12	13	12
hue, deg	x	17	30	26	17	x	x	11	25	36	16	9	34	11	15	16
satn index	x	41	30	51	47	x	x	52	20	13	14	44	11	61	49	43
Hunter parameters (constant abs)[d]																
L	69	50	51	53	56	49	53	58	55	52	56	51	62	56	55	56
a	50	45	31	39	44	46	47	50	41	38	46	44	37	63	63	65
b	20	11	19	22	14	4	2	6	18	19	15	6	19	−13	−14	−16
hue, deg	22	14	31	29	17	5	3	6	24	26	18	8	27	−12	−13	−14
satn index	53	46	36	45	46	46	47	50	45	43	48	45	41	64	64	66
total color diff[e]		22	26	19	15	26	24	18	16	20	14	23	14	38	39	41

[a] Calculated as grams of anhydrous citric acid per 100 g of sample. [b] Anthocyanin concentration calculated as cyanidin 3-glucoside, pH differential method used. [c] Anthocyanin concentration calculated as cyanidin 3-glucoside, single-pH method used. [d] Hunter values of the sample at $A(vis)_{max} = 1$, pH 3.5. [e] Total color difference when compared with Red FD&C 40. [f] Not determined; 1% solution is opaque.

the samples had undergone considerable polymerization. The exceptions are Welch's grape color extract and all of the cabbage colorants, which are very low in percent polymeric color. It has been suggested that acylated anthocyanins are more stable during storage than nonacylated anthocyanins (Sapers et al., 1981). The data in Table I tend to support this hypothesis as grape and cabbage are the only samples analyzed that contain acylated anthocyanins.

Tinctoral strength is commonly used to express coloring power and is often defined as the absorbance (optical density) at the visible wavelength of maximum absorbance (Clydesdale, 1978). The color of anthocyanin solutions is pH-dependent, the pigments exhibiting their maximum color intensity at about pH 1 and being nearly colorless at pH values of about 4.5 (Skrede, 1985). Listed in Table I are the tinctoral strength values for the samples in both water and in pH 1 buffer. In every case, the tinctoral strength is higher in pH 1 buffer than in distilled water. Comparison of the tinctoral strength data with the monomeric anthocyanin data shows that there is a direct relationship between monomeric anthocyanin and tinctoral strength at pH 1. Samples with high pigment concentrations exhibit high tinctoral strength values. Such a relationship does not hold for the monomeric anthocyanin data when it is compared with the tinctoral strength values for the pigments in distilled water. This is not surprising, as the range in pH for the samples varied from 3.18 to 4.35 (Table I). All of the samples showed at least a 2-fold increase in tinctoral strength when the pH 1 buffer was substituted for water except for the Obi-Pektin elderberry sample, which exhibited only a small increase. The low degree of spectral responsiveness to changes in pH in this sample indicates that the monomeric anthocyanins have undergone significant alteration during processing or storage (Wallin and Smith, 1977). This is also reflected in the high percent polymeric color value measured for the elderberry sample.

It should be emphasized that although tinctoral strength is commonly used to specify coloring power, it is not necessarily the most accurate measurement of coloring power. It has been noted that there can be a poor correlation between optical density and visual rank. This is especially true when comparisons are being made between different pigments (Clydesdale, 1978). The major reason is that optical density (absorbance) measurements at a single wavelength do not consider band width, as visual perception is analogous to the total area encompassed by the spectral curve (Clydesdale, 1978); hence, absorbance at a single wavelength will not necessarily reflect color intensity as visually observed.

Tristimulus colorimetry is perhaps the best method for estimating visual color. For cranberry juice cocktail, Hunter *L* and *a* values are highly correlated to anthocyanin concentration while Hunter *b* and Hunter hue show poor correlation to anthocyanin concentration as these functions are affected by browning components (Johnson et al., 1976). Two sets of Hunter tristimulus transmittance data are included in Table I. The first set shows the values for a 1% solution of the pigments in distilled water. At these concentrations, the Hunter data are of limited usefulness as the luminosity (*L*) values fall within the area of confusion, a region where changes in pigment concentration fail to correlate with changes in instrumental readings (Eagerman et al., 1973; Clydesdale, 1978).

Table II. Anthocyanidins of Commercial Colorants and Juice Concentrates

sample	% of anthocyanidins based on total peak area					
	delphinidin	cyanidin	petunidin	pelargonidin	peonidin	malvidin
elderberry[a]	NF[b]	100	NF	NF	NF	NF
black currant	47	53	NF	NF	NF	NF
blackberry	NF	100	NF	NF	NF	NF
blackraspberry	NF	100	NF	NF	NF	NF
Obi-Pektin Morello cherry[a]	NF	100	NF	NF	NF	NF
plum concentrate	NF	96	NF	NF	4	NF
Obi-Pektin bilberry	31	24	19	NF	8	18
Speda GSE[a]	32	15	18	NF	5	30
Welch's grape color extract	49	28	12	NF	4	7
San Red cabbage colorants[a,c]	NF	100	NF	NF	NF	NF

[a] Summary of results for all similar samples. [b] NF = not found. [c] Incomplete hydrolysis.

The second set of Hunter data compares the tristimulus values for the pigment solutions at constant absorbance (A (visible λ_{max} = 1) at pH 3.5 (Sapers et al., 1981). The values for Red FD&C 40 are included as a point of reference for comparison. All the samples exhibited lower L values than for Red FD&C 40, indicating that all of the colorants were darker than Red FD&C 40 (Skrede et al., 1983; Sapers et al., 1981). There was a large variation in the hue angle between the samples. When compared to Red FD&C 40, the Obi-Pektin elderberry, blackcurrant, two of the cherry, and the plum sample showed higher hue angles, indicating that these samples produced a more orange shade of red. The remaining samples had lower hue angles, especially the cabbage samples, indicating a less orange (more purple) shade of red. In terms of total color difference (ΔE), the blackberry concentrate, the plum concentrate, and the cherry samples were the closest in color to Red FD&C 40 while the cabbage colorant samples were the most dissimilar in color to Red FD&C 40.

Titratable acidity (TA) and pH are also listed as they are both influential factors in determining the color of anthocyanin solutions. Titratable acidity and pH values were similar for all of the samples except for blackcurrant, plum, Morello cherry 348, and the Spreda grape skin extract (GSE) samples, which were all high in titratable acidity, exceeding 7.0 g of anhydrous citric acid/100 g.

Individual Pigment Profiles by HPLC Separation/Photodiode Array Detection. *Blackcurrant.* The anthocyanins of the blackcurrant (*Ribes nigrum* L.) have been well characterized. Early work by Chandler and Harper (1962) identified the pigments of blackcurrant to be cyanidin and delphinidin 3-glucosides and 3-rutinosides. Later workers using thin-layer chromatography (Morton, 1968) and droplet countercurrent chromatography (DCCC) (Francis and Andersen, 1984) reported that the anthocyanins are present in the following relative amounts: delphinidin 3-rutinoside > cyanidin 3-rutinoside > delphinidin 3-glucoside > cyanidin 3-glucoside. Table II shows that cyanidin and delphinidin are the only anthocyanidins present in the blackcurrant sample analyzed. Figure 1 shows the HPLC chromatogram of the blackcurrant sample along with the relative peak areas. Spectral data and identification of the individual anthocyanins are included in Table III. Peak identification was made from comparison of retention times and spectral data to those of both authentic pigments from known sources and from literature values. Both the anthocyanidin and the anthocyanin profiles are consistent with the results of other workers.

Blackberry. The anthocyanins of blackberries along with other members (red and black raspberries, boysenberries, loganberries) of the *Rubus* species have been well

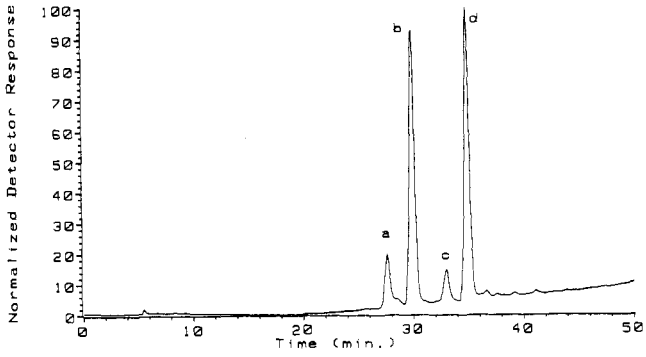

Figure 1. HPLC chromatogram of black currant anthocyanins. Peak area percentages: a, 9%; b, 44%; c, 5%; d, 42%.

studied (Jennings and Carmichael, 1980; Torre and Barritt, 1977; Barritt and Torre, 1973; Harborne and Hall, 1964). The anthocyanins in this family are characterized by cyanidin glycosides with pelargonidin glycosides present in some species. Additional glucose, xylose, or rhamnose residues may be present in various combinations to give di- or triglycosides (Jennings and Carmichael, 1980; Torre and Barritt, 1977). The anthocyanin profile of blackberries is characterized by cyanidin 3-glucoside and in some cases lesser quantities of cyanidin 3-rutinoside (Torre and Barritt, 1977; Barritt and Torre, 1973; Harborne and Hall, 1964). Pelargonidin 3-glucoside has been reported in one species (Sapers et al., 1986). Marion blackberries are unusual in that the major pigment is cyanidin 3-rutinoside (Barritt and Torre, 1973). Recently, Sapers et al. (1986), using HPLC, showed that, in addition to cyanidin 3-rutinoside and cyanidin 3-glucoside, thornless blackberries contained cyanidin 3-xyloside along with several acylated cyanidin derivatives tentatively identified as being acylated with a dicarboxylic acid. The anthocyanidin profile (Table II) for blackberry shows that cyanidin is the only aglycon present. An HPLC chromatogram (Figure 2) along with spectral data for each peak along with peak assignments is given in Table III. Both cyanidin 3-sophoroside and cyanidin 3-glucosylrutinoside have not been previously reported in blackberries. These pigments are, however, associated with red raspberries (Harborne and Hall, 1964).

There are several possible explanations for the presence of these unexpected pigments. The first is that the sample analyzed was a contaminated sample, possibly containing fruit of another species of *Rubus* (red raspberry or its clones). Alternatively, it is possible that these pigments are actually present in blackberries but have been previously unidentified. This is less likely as there has been a considerable amount of work in the characterization of blackberry anthocyanins. Further studies with authentic fruit are needed to determine whether these pigments are actually present in blackberries.

Table III. Spectral Characterization of Commercial Colorants and Juice Concentrates As Determined by HPLC/Photodiode Array Detection[a]

peak no.	identification	vis λ_{max}, nM	E_{440}/E_{vis}, %	acyl peak	acyl λ_{max}, nm	E_{acyl}/E_{vis}, %	10% aq KOH	borate treatment	method identifcn
			Black Currant Anthocyanins (Figure 1)						
a	dpd-3-glu	522	29	N			ND	ND	RT, UV
b	dpd-3-rut	525	31	N			ND	ND	RT, UV
c	cyd-3-glu	ND	ND	N			ND	ND	RT, UV
d	cyd-3-rut	517	35	N			ND	ND	RT, UV
			Blackberry Anthocyanins (Figure 2)						
a	cyd-3-soph	515	35	N			ND	ND	RT, UV
b	cyd-3-glurut	518	31	N			ND	ND	RT, UV
c	cyd-3-glu	515	33	N			ND	ND	RT, UV
d	cyd-3-rut	517	33	N			ND	ND	RT, UV
			Blackraspberry Anthocyanins (Figure 3)						
a	cyd-3-samb	515	37	N			ND	ND	RT, UV
b	cyd-3-glu and cyd-3-xylrut	519	33	N			ND	ND	RT, UV
c	cyd-3-rut	517	33	N			ND	ND	RT, UV
d	unidentified	ND	ND	ND	ND	ND	ND	ND	
			Elderberry Anthocyanins (Figure 4)						
a + b	cyd-3-samb-5-glu + cyd-3-glu-5-glu	514	19	N	ND		ND	ND	UV
c	cyd-3-samb	517	33	N	ND		ND	ND	RT, UV
d	cyd-3-glu	514	33	N	ND		ND	ND	RT, UV
e	unidentified	518	30	N	ND		ND	ND	
			Plum Anthocyanins (Figure 5)						
a	unidentified	ND	ND	ND	ND		ND	r	
b	cyd-3-glu	515	34	N			ND	r	RT, UV
c	cyd-3-rut	517	33	N			ND	r	RT, UV
d	unidentified	507	41	N			ND	r	
e	unidentified	506	45	N			ND	r	
f	unidentified	517	36	N			ND	r	
g	unidentified	508	45	N			ND	r	
			Cherry Anthocyanins (Figure 6)						
a	cyd-3-soph	517	28	N			nh	ND	RT, UV
b	cyd-3-glurut	519	33	N			nh	ND	RT, UV
c	cyd-3-glu	517	28	N			nh	ND	RT, UV
d	cyd-3-rut	517	32	N			nh	ND	RT, UV
			Bilberry Anthocyanins (Figure 7)						
a	unidentified	ND	ND	ND					
b	dpd-3-gal	523	33	N					
c	dpd-3-glu	524	34	N					
d	cyd-3-gal	516	34	N			ND	nr	RT, UV
e	ptd-3-gal	526	32	N			ND	nr	RT, UV
f	cyd-3-glu	517	37	N			ND	nr	RT, UV
g	ptd-3-glu (t)	526	35	N			ND	nr	UV
h	pet-3-glu (t)	527	36	N			ND	nr	UV
i	ND	ND	ND	ND	ND	ND	ND	r	
j	unidentified	ND	ND	ND	ND	ND	ND	r	
k	unidentified	ND	ND	ND	ND	ND	ND	r	
l	mvd-3-glu	527	30	N			ND	r	RT, UV
m	mvd-3-arab	527	35	N			ND	r	RT, UV
			Spreda GSE (Figure 8)						
a	dpd-3-glu	523	30	N			nh	nr	RT, UV
b	unidentified	ND	ND	ND			h	nr	
c	cyd-3-glu	517	34	N			nh	nr	RT, UV
d	ptd-3-glu	525	29	N			nh	nr	RT, UV
e	unidentified	ND	ND	ND			h	nr	
f	pnd-3-glu	517	35	N			nh	r	RT, UV
g	mvd-3-glu	527	29	N			nh	r	RT, UV
h	unidentified	ND	ND	ND	ND		h	r	
i	unidentified	515	41	N			nh	r	
j	unidentified	ND	ND	ND	ND		h	r	
k	unidentified	529	30	N			h	r	
l	unidentified	ND	ND	ND	ND		h	r	
m	unidentified	ND	ND	ND	ND		h	r	
n	unidentified	ND	ND	ND	ND		h	r	
o	unidentified	531	31	Y	310 sh	68	h	r	

Table III (Continued)

peak no.	identification	vis λ_{max}, nM	E_{440}/E_{vis}, %	acyl peak	acyl λ_{max}, nm	E_{acyl}/E_{vis}, %	10% aq KOH	borate treatment	method identificn
			Welch's Grape Colorant (Figure 9)						
a	unidentified	523	19	N			nh	r	
b	unidentified	523	22	N			nh	r	
c	dpd-3-glu	523	30	N			nh	nr	RT, UV
d	cyd-3-glu	515	33	N			nh	nr	RT, UV
e	ptd-3-glu	525	29	N			nh	nr	RT, UV
f	pnd-3-glu	516	35	N			nh	r	RT, UV
g	mvd-3-glu	527	30	N			nh	r	RT, UV
h	unidentified	527	30	N			h	r	
i	unidentified	520	35	N			h	r	
j	unidentified	527	32	N			h	r	
k	unidentified	531	19	Y	305	62	h	r	
l	unidentified	522	20	Y	313	60	h	r	
m	unidentified	533	17	Y	301 sh	68	h	r	
n	unidentified	532	31	Y	317	71	h	r	
o	unidentified	528	19	Y	300 sh	66	h	r	
p	unidentified	522	32	Y	313 sh	72	h	r	
q	unidentified	531	28	Y	310 sh	71	h	r	
r	unidentified	532	31	Y	313 sh	75	h	r	
			Red Cabbage Colorant (Figure 10)						
a	unidentified	509	24	N			nh	nr	
b	unidentified	529	21	Y	333	58	h	r	
c	unidentified	523	16	Y	310 sh	64	h	r	
d	unidentified	519	15	Y	323	69	h	r	
e	unidentified	534	18	Y	310 sh	128	h	r	
f	unidentified	533	20	Y	327	111	h	r	
g	unidentified	523	18	Y	315	59	h	r	
h	unidentified	523	18	Y	329	53	h	r	
i	unidentified	532	24	Y	327	98	h	r	
j	unidentified	525	23	Y	327	98	h	r	
k	unidentified	537	18	Y	320	100	h	r	
l	unidentified	536	18	Y	331	114	h	r	
m	unidentified	532	24	Y	327	103	h	r	
n	unidentified	535	35	Y	331	121	h	r	
z	unidentified	515	33	N			nh	r	

[a] Key: ND = not determined; sh = shoulder; Y = acylation with hydroxy aromatic acid detected; N = no acylation with hydroxy aromatic acid detected; nh = not hydrolyzed by alkali treatment; h = hydrolyzed by alkali treatment; nr = not retained in C_{18} column after elution with borate buffer; r = retained in C_{18} column after elution with borate buffer; UV = identification based on UV–visible spectral data; RT = identification based on comparison of retention time with pigment standard; (t) = tentative identification. Key to pigment identifications: cyd = cyanidin; arab = arabinoside; dpd = delphinidin; gal = galactoside; mvd = malvidin; glu = glucoside; pgd = pelargonidin; glurut = glucosylrutinoside; pnd = peonidin; rut = rutinoside; ptd = petunidin; samb = sambubioside; soph = sophoroside; xylrut = xylosylrutinoside.

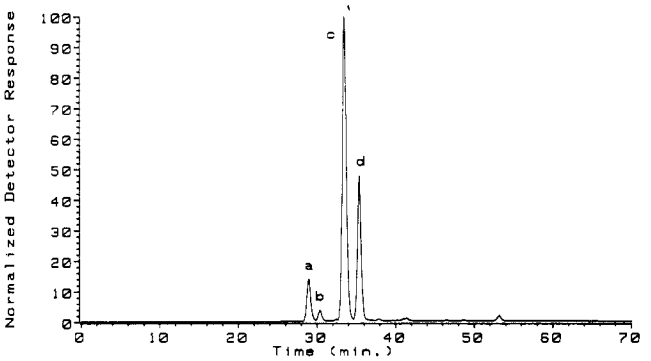

Figure 2. HPLC chromatogram of blackberry anthocyanins. Peak area percentages: a, 9%; b, 4%; c, 57%; d, 30%.

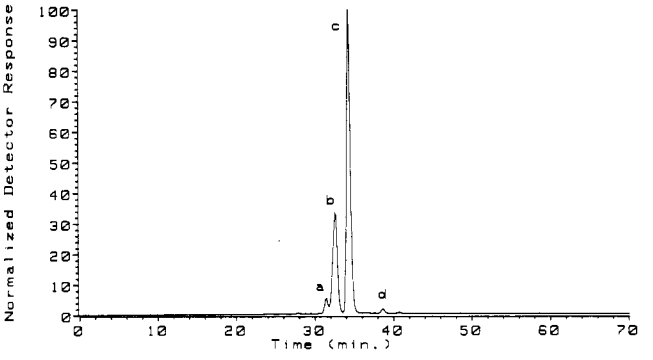

Figure 3. HPLC chromatogram of blackraspberry anthocyanins. Peak area percentages: a, 3%; b, 33%; c, 64%; d, trace.

Black Raspberry. Unlike the blackberry, black raspberries (*Rubus occidentalis, Rubus leucodermis*) are characterized by the presence of xylose-containing pigments. The four anthocyanins of black raspberry are cyanidin 3-glucoside, cyanidin 3-rutinoside, cyanidin 3-sambubioside, and cyanidin 3-xylosylrutinoside (Harborne and Hall, 1964; Nybom, 1968; Barritt and Torre, 1973; Torre and Barritt, 1977). A previous report indicates that the 3-xylosylrutinoside and the 3-rutinoside are generally present in the largest quantities (Torre and Barritt, 1977). Analysis of anthocyanidins in black raspberry (Table II) shows that cyanidin is the only aglycon present. The HPLC anthocyanin profile is given in Figure 3. Spectral information of the individual peaks is given in Table III. Black raspberry anthocyanins were separated by preparative paper chromatography in AHW (acetic acid/HCl/water, 15/3/82). Four bands of pigments were found, corresponding to the four anthocyanins previously reported for black raspberry. The darkest bands corresponding to the 3-rutinoside and to the 3-xylosylrutinoside. The band (R_f 0.73) corresponding to cyanidin 3-xylosylrutinoside (Harborne, 1967) was isolated and then chromatographed by HPLC. The retention of cyanidin 3-xylosylrutinoside was found to be the same as that of cyanidin

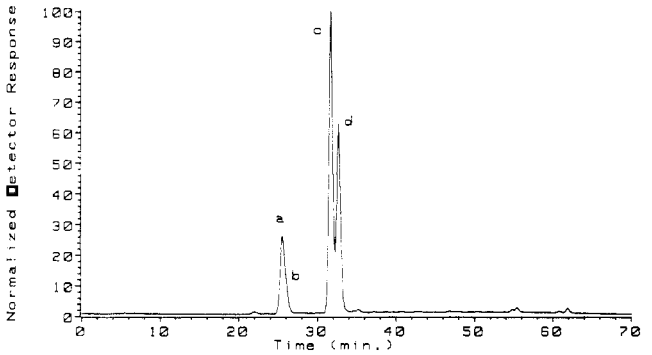

Figure 4. HPLC chromatogram of elderberry anthocyanins. Peak area percentages follow. San Red ELF: a + b, 31%; c, 42%; d, 27%. Elderberry 200: a + b, 37%; c, 37%; d, 27%.

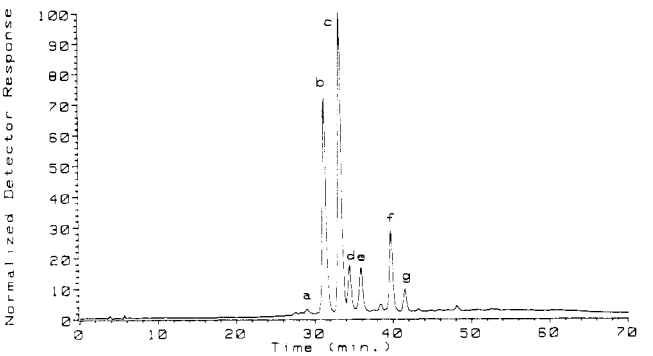

Figure 5. HPLC chromatogram of plum anthocyanins. Peak area percentages: a, less than 1%; b, 37%; c, 45%; d, 3%; e, 3%; f, 12%; g, trace.

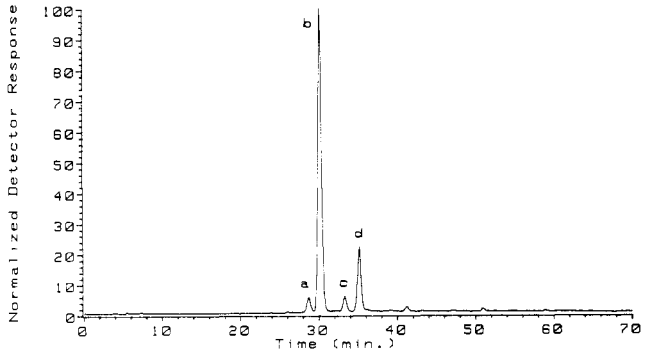

Figure 6. HPLC chromatogram of Morello cherry anthocyanins. Peak area percentages of Morello Cherry 240: a, less than 1%; b, 65%; c, 14%; d, 14%. Peak area percentages of Morello Cherry 240D: a, 3%; b, 74%; c, 3%; d, 16%. Peak area percentages of Morello Cherry 348: a, 3%; b, 72%; c, 4%; d, 15%.

3-glucoside. This elution time is later than one might anticipate for a triglycoside. One would normally expect triglycosides to elute sooner than diglycosides, which in turn elute before monoglycosides. The late elution of cyanidin 3-rutinoside suggests that the hydrophobic methyl group of rhamnose causes increased retention (Spanos and Wrolstad, 1987). Sapers et al. (1986) suggested that cyanidin 3-xyloside would be more hydrophobic than either cyanidin 3-glucoside or cyanidin 3-rutinoside. Thus, the hydrophobicity of both rhamnose and xylose probably account for the late retention of cyanidin 3-xylosylrutinoside.

From a chromatographic standpoint, samples suspected of containing both cyanidin 3-glucoside and cyanidin 3-xylosylrutinoside will need to be separated on an alternate chromatographic system capable of resolving this pair.

The anthocyanin profile for black raspberry is consistent with previous reports for this species. Determination of the proportion of the major pigment was not possible due to coelution of cyanidin 3-glucoside with cyanidin 3-xylosylrutinoside.

Elderberry. The anthocyanin profile of elderberry (*Sambucus nigra* L.) has been previously characterized by HPLC. The major anthocyanins along with their relative percentages (based on HPLC peak area) have been reported to be cyanidin 3-glucoside (65.7%) and cyanidin 3-sambubioside (32.4%). The two minor pigments are cyanidin 3-sambubioside 5-glucoside (1.1%) and cyanidin 3-glucoside 5-glucoside (0.8%) (Bronnum-Hansen and Hansen, 1983). Table II shows that cyanidin is the only aglycon present in both samples of elderberry analyzed. Figure 4 shows the anthocyanin profile for elderberry along with the peak area percentage for both of the elderberry samples analyzed. Spectral information and peak identification of the individual peaks are given in Table III.

Plum. In a survey of 15 common plum (*Prunus domestica*) varieties, Harborne and Hall (1964) reported the major anthocyanins to be cyanidin 3-glucoside and cyanidin 3-rutinoside accompanied by traces of the related peonidin derivatives. Tables II and III list the HPLC data for plum. The major aglycon is cyanidin with trace amounts of peonidin. Figure 5 shows the HPLC chromatogram of the plum anthocyanins. Peaks b and c correspond to cyanidin 3-glucoside and cyanidin 3-rutinoside, respectively, based on previously established retention times and spectral data (Hong and Wrolstad, 1990). Peak a is present in insufficient quantity for spectral determination. On the basis of anthocyanidin data (Table II) and its retention characteristics (more polar than cyanidin 3-glucoside), it is likely to be either cyanidin 3-galactoside or a cyanidin di- or triglycoside. Peak f shows spectral properties indicative of a peonidin derivative and is identical in retention time with that of peonidin 3-glucoside from cranberries (Hong and Wrolstad, 1990). The fact that it is concentrated in the Sep-Pak after treatment with alkaline borate is additional evidence that this is a peonidin glycoside. Peaks d, e and g have spectral characteristics that do not correspond with any of the previously identified anthocyanins. They all are selectively concentrated on the Sep-Pak after elution with alkaline borate buffer.

Sour Cherry. The anthocyanin profile of sour cherries (*Prunus cerasus*) is complex (Timberlake and Bridle, 1982). Harborne and Hall (1964) reported cyanidin 3-glucosylrutinoside, cyanidin 3-sophoroside, cyanidin 3-rutinoside, and cyanidin 3-glucoside in six different varieties of sour cherries; the Morello A variety was reported to contain only cyanidin 3-glucoside and cyanidin 3-glucosylrutinoside. Peonidin 3-glucoside and peonidin 3-rutinoside were reported as minor pigments in this same variety (Dekazos, 1970). Later work by Shrikhande and Francis (1973) showed that cyanidin 3-xylosylrutinoside was a minor component in the Montmorency variety of sour cherries. Previous reports of cyanidin 3-gentiobioside in sour cherries are incorrect (Du et al., 1975).

Figure 6 shows the anthocyanin profile of Morello cherry. All three of the cherry samples showed similar profiles. The spectral data for the cherry anthocyanins are shown in Tables II and III.

Bilberry. The bilberry or whortleberry (*Vaccinium myrtillus*) is a native of parts of Europe and northern Asia (Timberlake and Bridle, 1982). Suomalainen and Keranen (1961) reported that this species contains cyanidin, delphinidin, petunidin, and malvidin glucosides and

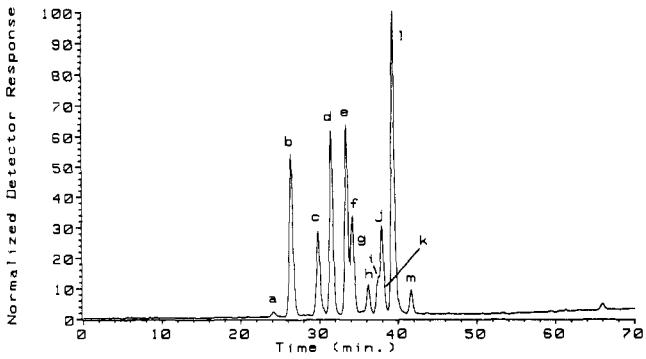

Figure 7. HPLC chromatogram of bilberry anthocyanins. Peak area percentages: a, trace, less than 1%; b, 19%; c, 9%; d, 15%; e, 14%; f, 9%; g, trace; h, 2%; i + j, 9%; k, trace; l, 22%; m, 1%.

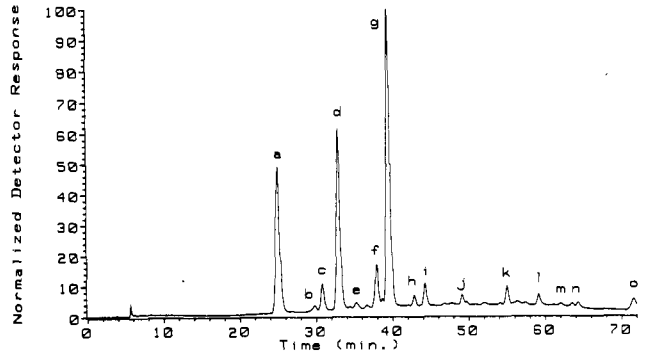

Figure 8. HPLC chromatogram of Spreda GSE anthocyanins. Peak area percentages of Spreda GSE: a, 17%; c, 3%; d, 21%; f, 7%; g, 43%; k, 4%; o, 2%. The remaining peaks were present in quantities less than 1%. Peak area percentages of Spreda GSE 501: a, 15%; c, 3%; d, 17%; f, 6%; g, 45%; k, 3%; o, 7%. The remaining peaks were present in quantities less than 1%.

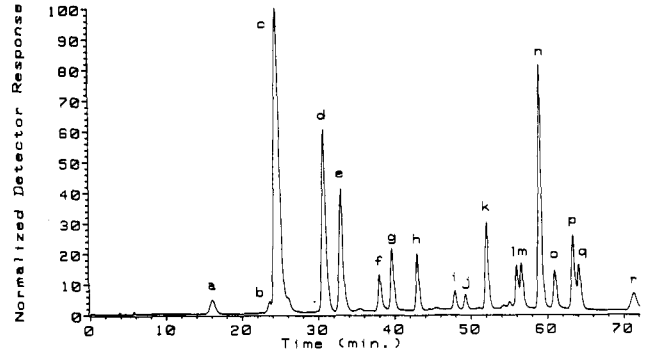

Figure 9. HPLC chromatogram of the anthocyanins in Welch's grape colorant. Peak area percentages: a, 2%; b, 1%; c, 31%; d, 16%; e, 10%; f, 3%; g, 5%; h, 3%; i, 1%; j, trace, less than 1%; k, 5%; l, 5%; m, 9%; n, 3%; o, 2%.

arabinosides. Later work by Baj et al. (1983) using both HPLC and gas–liquid chromatography (GLC) found all possible combinations of cyanidin, delphinidin, petunidin, peonidin, and malvidin 3-galactosides, 3-glucosides, and 3-arabinosides in bilberry. Quantitatively, the delphinidin glycosides were present in the largest quantities, and the peonidin glycosides were the least abundant. Since the bilberry is closely related to the lowbush blueberry (*Vaccinium angustifolium*), it is likely that their anthocyanin patterns are similar (Timberlake and Bridle, 1982). It is appropriate then to include a brief discussion of the anthocyanins of the various blueberry species. Francis et al. (1966), found that the ripe fruits of the lowbush blueberry contained all possible combinations of delphinidin, malvidin, petunidin, peonidin, and cyanidin 3-galactosides, 3-glucosides, and 3-arabinosides. The glucosides were found to be present in much greater quantity than the galactosides. Only trace quantities of the arabinosides were found. Diglycosides were also detected but not identified. A review of the anthocyanins of another related species, the highbush blueberry (*Vaccinium corymbosum*), shows that this species contains the same anthocyanins as the lowbush blueberry but in different proportions (Timberlake and Bridle, 1982). Recently, Sapers et al. (1984) in a study of lowbush blueberry separated as many as 16 anthocyanins by HPLC. Three distinct anthocyanin patterns were found for the 11 different cultivars studied. The anthocyanins of a related species, the bog whortleberry (*Vaccinium uliginosum* L.) was also found to contain all possible combinations of the previously mentioned pigments, with malvidin 3-glucoside as the predominant pigment (Andersen, 1987).

Figure 7 shows the anthocyanin profile for the bilberry colorant sample while Tables II and III list the spectral data and tentative identifications for each of the peaks.

Grape. Pigment from wine grape skins (*Vitis vinifera*) has been available commercially for some time (Francis, 1975). The anthocyanin composition of grapes has been well studied. So specific are the pigment profiles of grapes that differentiation of the various species of *Vitis* are possible through the study of the anthocyanin composition (Ribereau-Gayon, 1982). The anthocyanins of the *vinifera* species are based largely on malvidin, to lesser extents on delphinidin, petunidin, and peonidin, and only to a small extent on cyanidin. They are found not only as the 3-glucosides but also as 3-glucosides acylated with acetic acid, *p*-coumaric acid, and caffeic acid (Bakker and Timberlake, 1985; Wulf and Nagel, 1978). Numerous works have been published involving the characterization of grape anthocyanins, and the specific results of those studies have been summarized by Ribereau-Gayon (1982).

Figure 8 shows the HPLC chromatogram for Spreda GSE. Spectral data for the major peaks are given in Table III. The anthocyanidin analysis (Table II) shows that this sample contains all of the common anthocyanidin aglycons except for pelargonidin. Malvidin and delphinidin are the major anthocyanidins present.

The other sample of grape colorant, Welch's grape color extract, is specified by the manufacturer to be "A natural color pigment extracted from Concord Grape (*Vitis labrusca* var. Concord) using citric acid". A review specific for the anthocyanins for the Concord variety of *Vitis labrusca* show that the major pigment is delphinidin 3-glucoside followed by (listed in descending order by relative quantity) the 3-glucosides of cyanidin, petunidin, malvidin, and peonidin. Acylations with *p*-coumaric acid and 3,5-diglucosides of cyanidin and delphinidin have also been reported (Wrolstad, 1976; Ingalsbe et al., 1963; Ohta et al., 1979). A chromatogram of this extract is shown in Figure 9 while spectral data and peak identification are given in Tables II and III.

Red Cabbage. The usefulness of anthocyanin pigment preparations from red cabbage (*Brassica oleracea*) has been discussed in detail (Sapers et al., 1981; Shewfelt and Ahmed, 1977, 1978). Red cabbage, in common with other cruciferous plants, has a very sophisticated pattern of acylated anthocyanins. This complexity is reflected in the many differing reports regarding the exact composition of the red cabbage anthocyanins (Timberlake and Bridle, 1982). The definitive work for red cabbage

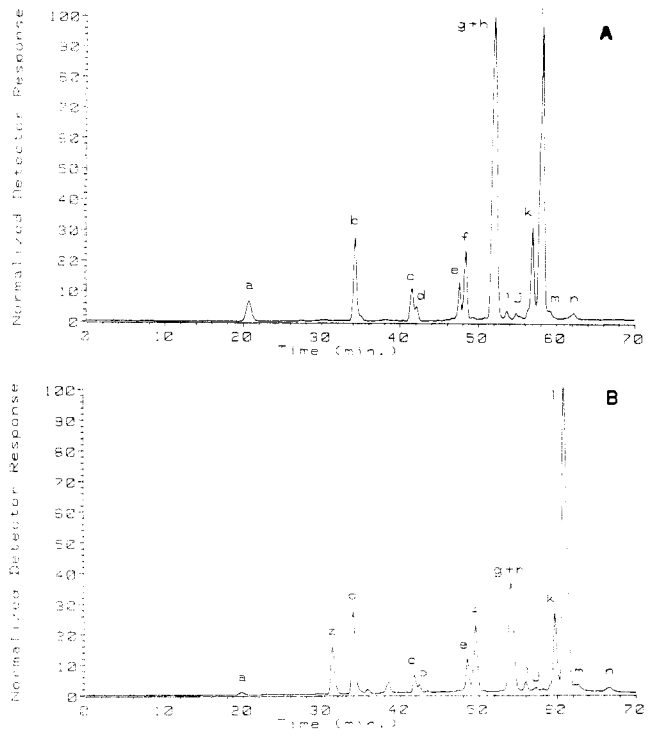

Figure 10. HPLC chromatogram of red cabbage anthocyanins: A, San Red RC powder, untreated; B, San Red RC liquid, untreated. Peak area percentages of San Red RC powder: a, 3%; z, not found; b, 7%; c + d, 4%; e, 3%; f, 5%; g + h, 36%; i, trace, less than 1%; j, trace; k, 8%; l, 31%; m, trace; n, trace. Peak area percentages of San Red RCF: a, 3%; z, not found; b, 9%; c + d, 7%; e, 3%; f, 5%; g + h, 42%; i, trace, less than 1%; j, trace; k, 8%; l, trace; m, trace; n, trace. Peak area percentages of San Red RC liquid: a, trace; z, 6%; b, 10%; c + d, 2%; e, 2%; f, 7%; g + h, 16%; i, trace; j, trace; k, 10%; l, 43%; m, trace; n, trace.

anthocyanin composition is by Hrazdina et al. (1977). What follows is a summary of their findings: Eight anthocyanins were isolated and characterized. They were all identified as derivatives of cyanidin 3-sophoroside 5-glucoside. In addition to cyanidin 3-sophoroside 5-glucoside, acylation was found at the position 3 sugars by the following groups: malonic acid, *p*-coumaric acid in both 1 and 2 mol ratios, ferulic acid in both 1 and 2 mol ratios, and sinapic acid in both 1 and 2 mol ratios. In the case of acylation with more than one acid, mixed acylation (where two different acylating acids occur in the same anthocyanin) does not occur (Hrazdina et al., 1977). Recently, new acylated forms of the red cabbage pigments have been characterized, including the discovery of acylation with glucose derivatives of ferulic and *p*-coumaric acids to the sophoroside sugar moiety (Idaka et al., 1987; Itaka, 1986).

Shown in Figure 10A is a typical chromatogram of the anthocyanins in either the San Red RCF or San Red RC powder red cabbage samples. The spectral properties of the individual peaks are listed in Table III. The adsorption of the pigments onto PVPP was omitted in the sample preparation steps (Hong and Wrolstad, 1990) as these pigments did not adsorb well to the PVPP, resulting in a severe loss of pigments. The anthocyanidin profile (Table II) for red cabbage shows that cyanidin is the only aglycon present. Several early-eluting peaks were present in the anthocyanidin chromatogram, most probably the result of incomplete acid hydrolysis. The early-eluting peaks remained in the chromatogram even after the red cabbage anthocyanins were subjected to acid hydrolysis for twice the normal hydrolysis time. This attests to the high stability of acylated anthocyanins (Sapers et al., 1981).

Peaks a–m exhibit E_{440}/E_{vis} ratios indicative of 3,5-glycosidation. Peak a is the only peak that shows no acylation by an aromatic organic acid. Its very early elution time indicates that it may be either cyanidin 3-sophoroside 5-glucoside or cyanidin 3-malonylsophoroside 5-glucoside as reported by Hrazdina et al. (1977). Peaks b–d, g, and h show E_{acyl}/E_{vis} ratios in the 50–70% range, indicating acylation with 1 mol of the acylating acid. The nature of the acylating hydroxy aromatic acid can be determined by the absorption spectrum in the 300–340-nm range. Acylated anthocyanins containing sinapic and ferulic acids both have UV acyl absorption maxima (in acidified methanol) in the 330-nm range whereas anthocyanins acylated with *p*-coumaric acid exhibit a lower UV maximum at 310 nm (Hrazdina, 1977). All of the spectral information indicates that peaks b, d, and h are derivatives of cyanidin 3-sophoroside 5-glucoside monoacylated with either sinapic or ferulic acid. Similarly, peaks c and g are derivatives of cyanidin 3-sophoroside 5-glucoside monoacylated with *p*-coumaric acid.

Peaks e, f, and i–m have E_{acyl}/E_{vis} ratios of 90–120%, indicating acylation with 2 mol of the acylating acid. The acyl UV maximum for peak e shows that it is likely to be a bis(*p*-coumaric acid)-acylated derivative of cyanidin 3-sophoroside 5-glucoside. Similarly, higher acyl wavelength maxima for peaks f and i–m show that these peaks are likely to be bis(sinapic acid) or bis(ferulic acid) derivatives of cyanidin 3-sophoroside 5-glucoside.

The latest eluting peak (n) exhibits an E_{440}/E_{vis} ratio indicative of a 3-glycoside. The E_{acyl}/E_{vis} ratio shows that the peak is diacylated. The acyl wavelength maximum indicates that acylation is likely to be with either sinapic acid or ferulic acid. From this information, the late retention time for this peak is not unexpected. The presence of acylated derivatives of cyanidin 3-sophoroside 5-glucoside is well established in red cabbage (Timberlake and Bridle, 1982), and there are no reports of acylated 3-glycosides. One possibility is that this peak is an acylated derivative of cyanidin 3-sophoroside arising from hydrolysis of the cabbage anthocyanins.

All of the anthocyanins in red cabbage were found to be cyanidin (Table II), which agrees with previous findings. Inspection of the visible wavelength maxima of the red cabbage anthocyanins show that the visible wavelength maximum of cyanidin 3-sophoroside 5-glucoside is 515 nm, a figure consistent with our previous observations of cyanidin glycosides. The acylated pigments, however, tended to have a much higher wavelength maximum, even higher than for the delphinidin derivatives. Previous studies with anthocyanins in acidified methanol showed only small (1–5-nm) differences in the visible wavelength maxima between acylated and nonacylated pigments for the same aglycon (Hrazdina et al., 1977; Wulf and Nagel, 1978). These results show that, in a predominantly aqueous HPLC solvent, the visible wavelength maximum for acylated cyanidin glycosides is very different from that of nonacylated cyanidin glycosides. The difference in some cases is by as much as 20 nm. For example, peak b, identified as a monoacylated derivative of cyanidin 3-sophoroside 5-glucoside, has a retention time similar to that of cyanidin 3-glucoside, yet the wavelength maximum for this peak is 529 nm, 14 nm higher than for cyanidin 3-glucoside. From these observations, it appears that acylation (with hydroxy aromatic acids) of the anthocyanins produces a shift to a higher visible wavelength maximum when spectra are measured in the

aqueous HPLC solvent used. Additional support for this hypothesis is that diacylated anthocyanins have a higher visible wavelength maximum than monoacylated anthocyanins. This could account for the large difference in Hunter values for the red cabbage samples when compared to the other colorants examined. In light of these findings, when acylation with cinnamic acids is suspected, the visible λ_{max} will not be an accurate indicator of the nature of the aglycon. Further studies are needed to establish more precisely the effect of acylation with cinnamic acids on the spectra of anthocyanins in aqueous solvent systems.

In regard to the anthocyanin profile of the three different samples of red cabbage colorants, the San Red RC powder and the San Red RCF samples were found to have very similar pigment profiles. The San Red RC liquid sample (Figure 10B) was different from the other two samples in that an extra early-eluting peak (z) was present and that the major pigment was peak 1. Peak z exhibits spectral characteristics corresponding to that of a cyanidin derivative.

CONCLUSIONS

In this paper we demonstrate the utility of HPLC/photodiode array detection as a valuable tool for characterization of anthocyanins. The spectral characteristics of the anthocyanins yield useful information in regard to the nature of the aglycon and the sugar substitution pattern, while the retention characteristics on reversed-phase HPLC yield information as to the nature of the sugar moieties. The behavior of the anthocyanins after alkaline hydrolysis and after selective elution from a reversed-phase cartridge with borate buffer gives additional indices useful for characterizing these pigments. These methods are particularly useful for assignment of peak identities in materials for which the anthocyanins have been previously identified. The information presented provides color indices for a variety of potentially useful colorants along with analytical information that can be used to identify the presence of these colorants.

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Use of HPLC Separation/Photodiode Array Detection for Characterization of Anthocyanins†

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A systematic procedure for separation and characterization of anthocyanins is described. Separation of pigments was achieved by high-performance liquid chromatography (HPLC) on a polymer reversed-phase column. Methods for preparation of an anthocyanin isolate free of other interfering phenolics were developed. Photodiode array detection was employed to determine the UV–visible spectral characteristics of the pigments. Derivatives of delphinidin (delphinidin, petunidin, malvidin) can be distinguished from derivatives of cyanidin (cyanidin, peonidin), which in turn can be distinguished from pelargonidin derivatives on the basis of their different UV–visible spectra. Acylation with cinnamic acids and differentiation between 3- and 3,5-glycosidation can also be determined from the UV–visible spectrum. Auxiliary sample preparation techniques useful for pigment characterization included alkaline hydrolysis of the anthocyanins for determination of acylation. Anthocyanins not containing an o-diphenolic system can be enriched on a C_{18} reversed-phase cartridge by elution with alkaline borate buffer. With a combination of these techniques, peak assignments for the anthocyanins from sources whose anthocyanin composition is known can be readily made.

The anthocyanins are the natural pigments responsible for the red, blue, and purple colors of many plants. There are many different anthocyanins found in nature. The individual anthocyanin composition for any given plant is distinctive, and the analysis of anthocyanin and/or other flavonoids has therefore been very useful in distinguishing between species. This is especially true if there are qualitative differences (Stewart et al., 1979). With more closely related plants, such as those differing

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