Isolation and Stabilization of Anthocyanins from Tart Cherries (*Prunus cerasus* L.)

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Anthocyanin pigments from Montmorency and English Morello tart cherry (*Prunus cerasus* L.) cultivars and three Michigan State University (MSU) tart cherry hybrids were stabilized with phosphoric acid, maltodextrin, and α- and β- cyclodextrins. The anthocyanin levels in the stored juice samples were analyzed by HPLC and are expressed as the sum of their peak areas. A novel method has been developed for the isolation of anthocyanin pigments from Montmorency, English Morello, and the MSU hybrid II 9 (11). These pigment mixtures, when stored as powders with dextrins, were found to be stable at room temperature under laboratory conditions even after 12 weeks. Also, at 12 weeks, the powdered anthocyanins without dextrins showed less degradation than those in solution.

INTRODUCTION

There is considerable interest in the development of food colorants from natural sources to replace synthetic food colorants. The role of anthocyanins as food coloring agents becomes very important since they are universally associated with attractive, colorful, and flavorful fruits (Francis, 1989). We have reported earlier the methods for extraction and separation of the anthocyanins from tart cherries, *Prunus cerasus* L. (Chandra et al., 1992). The presence of cyanidin 3-sophoroside, cyanidin 3-glucosylrutinoside, cyanidin 3-glucoside, cyanidin 3-rutinoside, and peonidin 3-galactoside was established in Montmorency, English Morello, and the Michigan State University (MSU) hybrid selections II 7 (30), I 21 (33), and II 9 (11) (Chandra et al., 1992).

The major problem associated with the storage of anthocyanins is their unstability, caused by certain enzymes, pH, temperature, oxygen, and light (Francis, 1989). Colorless flavonoids and polyphenols are frequently found in association with the anthocyanins in the vacuoles of higher plants (Brouillard et al., 1989). It has been suggested that various phenolics coexisting with the anthocyanins were more susceptible to oxidative degradation, which in turn accelerates the degradation of anthocyanins (Van Buren et al., 1960; Seigel et al., 1971; Pifferi and Cultrera, 1974). Anthocyanases, polyphenol oxidases (PPO), and peroxidases are also implicated for contributing to anthocyanin unstability (Wissemann and Lee, 1980). However, stability of anthocyanins is known to be increased by copigmentation with other polyphenolics coexisting in the same system (Sweeney et al., 1981; Mazza and Brouillard, 1987; Brouillard et al., 1989). Molecules that can copigment with anthocyanins include flavonoids, polyphenols, alkaloids, amino acids, and organic acids. The copigmentation effect exists only in aqueous solutions and is sensitive to pH, temperature, and composition of the solution. Copigmentation protects anthocyanins against hydration, thus preserving their red color (Brouillard et al., 1989).

Isolation and stabilization of these natural pigments from tart cherry cultivars are important for their potential use as natural food colorants. Various European patents have been issued on the isolation of pigments from fruits. It has been reported that the pigments from grape juice were processed by adsorbing them on a resin and subsequently eluting the color with ethanol (Francis, 1989). In another process, finely divided oxides such as silicic acid, titanium oxide, or aluminum oxide suspended in CH_2Cl_2 solution containing dissolved polystyrene were used to process anthocyanins from aqueous solutions (Mirabel and Meiller, 1978). The MSU tart cherry selections gave higher pigment levels than the popular commercial variety Montmorency (Chandra et al., 1992). A rapid and economical method for the isolation of anthocyanin pigments from tart cherries and their relative stabilities in solution or as a powder under laboratory conditions for 12 weeks are discussed.

MATERIALS AND METHODS

Isolation of Pigments from Tart Cherries. XAD-2 resin (amberlite, mesh size 20–50; Sigma Chemical Co.) was soaked in EtOH (500 mL) for 10 min and washed with EtOH (1000 mL) in a medium-porosity sintered funnel under vacuum. It was then slurry-packed in a glass column (33 × 6 cm, bed volume 900 mL). The column was then washed with distilled water (2.7 L) to replace EtOH. Montmorency cherry juice from homogenized cherries including the skin (50 mL) was centrifuged (10000*g*, 10 min, 4 °C) to remove insoluble materials and applied to the column. The column was washed with H_2O (9 L) until the colorless washings gave a pH of about 7. The adsorbed pigments were then eluted with EtOH (600 mL). The red ethanolic solution was concentrated at 50 °C *in vacuo*, and the aqueous solution was lyophilized to afford an amorphous, nonhygroscopic red powder. Similarly, pigments were isolated from English Morello and II 9 (11) cherry samples. The amounts of pigment isolates obtained from various tart cherry selections were 100, 450, 650, 40, 100, 70, and 600 mg from Montmorency single-strength juice (50 mL), Montmorency juice concentrate (50 mL), Montmorency juice concentrate (50 mL) diluted with H_2O (50 mL), Montmorency homogenized cherry juice (grams per milliliter, 50 mL), II 9 (11) homogenized cherry juice (grams per milliliter, 50 mL), English Morello homogenized cherry juice (grams per milliliter, 50 mL), and English Morello concentrate (50 mL), respectively.

Stability of Cherry Juice and Isolated Anthocyanins with Dextrins. Pitted cherries (10 g each) were homogenized with H_2O (5 mL) and centrifuged (10000*g*, 10 min). The supernatant was decanted (4 mL) and divided into four 1-mL portions. To each 1-mL juice sample were added separately 25 mg each of α-cyclodextrin (αCD), β-cyclodextrin (βCD) (Pharmatech Inc., Alachua, FL), and maltodextrin (MD) (Staley, Decatur, IL) as solutions in 1 mL of H_2O each and 1 mL of H_2O. All samples were filtered through a 0.22-μm filter. The anthocyanin powder from Montmorency, English Morello, and II 9 (11) (80 mg each) was dissolved in water (8 mL each) and divided into 2-mL aliquots. αCD, βCD, and MD (100 mg each in 2 mL of H_2O) and 2 mL of

0021-8561/93/1441-1062$04.00/0

Table I. Relative Anthocyanin Concentrations in Various Tart Cherry Juices Stored in Aqueous Phosphoric Acid Expressed as the Sum of Actual Peak Areas

cherry selection	% of H_3PO_4	weeks 0	2	12
Montmorency	4	2.53	2.22	1.72
	1	2.68	2.35	1.18
	0	2.17	1.13	0.20
English Morello	4	14.34	14.07	12.51
	1	13.28	10.25	9.30
	0	11.46	8.69	5.42
II 9 (11)	4	39.48	35.99	26.68
	1	36.40	34.06	25.30
	0	35.52	32.28	9.64
I 21 (33)	4	20.74	19.32	10.73
	1	18.91	16.57	11.79
	0	15.91	13.04	4.02
II 7 (30)	4	16.80	13.74	7.68
	1	16.25	14.52	11.00
	0	14.07	9.74	0.21

H_2O were added separately to the four aliquots. The resulting solutions were further divided into 2-mL portions each (eight portions of 2 mL each). One set was lyophilized, and the remaining solutions were filtered through a 0.22-μm filter. The juice samples from Montmorency, English Morello, II (9) 11, II (7) 30, and I (21) 33 and the anthocyanins isolated from Montmorency, English Morello, and II (9) 11 were stored with MD and α- and βCD at ambient conditions exposed to laboratory light (10 h) each day for 12 weeks prior to analysis.

Stability of Cherry Juice with H_3PO_4. Cherry juice were prepared (grams per milliliter) from Montmorency, English Morello, II 9 (11), II 7 (30), and I 21 (33). To these juice samples was added 400, 100, and 0 μL of phosphoric acid (85%) to obtain a final volume of 10 mL each, respectively. The pH values of samples containing 4, 1, and 0% H_3PO_4 were 1.20, 1.75, and 3.9, respectively. The juice samples were filter-sterilized (0.22 μm) and stored similarly to the samples containing dextrins.

HPLC Analyses. The HPLC analyses were carried out at 0-, 2-, and 12-week intervals. The results are shown in Tables I–IV. The analyses was carried out as reported earlier (Chandra et al., 1992). The anthocyanin concentrations are expressed as the sum of actual peak areas of anthocyanins A1–A5 (Tables I–IV) for each sample (Chandra et al., 1992). The concentrations of the anthocyanins from the HPLC analyses at 0 time (Tables I–IV) were used to calculate the percentages of the anthocyanins at the end of 12 weeks under different storage conditions.

RESULTS AND DISCUSSION

Anthocyanins prepared from Montmorency, English Morello, and the MSU hybrid selection II 9 (11) cherry juices were red amorphous powders with a bitter taste. Isolation of the anthocyanins using XAD-2 resin suggested that 50 mL of the Montmorency juice concentrate diluted with 50 mL of water is the optimum quantity that could be loaded on a 33 × 6 cm column containing 300 g of XAD-2 resin. The homogenized cherry juice from MSU selection II 9 (11) afforded 2.5 times more pigment mixture than that obtained from a similar amount of Montmorency cherries.

After 12 weeks of storage, the juices without any H_3PO_4 showed a decrease in the red color, while Montmorency and II 7 (30) were completely degraded to brown. A small amount of brown precipitation was observed in all samples with relatively lesser amounts in the samples containing H_3PO_4. The HPLC analysis of juice samples stored in 4% H_3PO_4 clearly showed less degradation of anthocyanins (Table I). However, English Morello was found to retain 47% of the original anthocyanins without H_3PO_4. The stabilization of the anthocyanins from tart cherry selections

Table II. Relative Anthocyanin Concentrations in Various Tart Cherry Juices Stored with Dextrins Expressed as the Sum of Actual Peak Areas

cherry selection	stabilizer[a]	weeks 0	2	12
Montmorency	MD	4.21	1.66	1.35
	βCD	4.11	2.79	2.00
	αCD	3.49	1.82	1.75
	–	3.85	1.05	0.00
English Morello	MD	11.48	6.62	3.67
	βCD	11.89	8.10	4.39
	αCD	12.07	4.98	2.37
	–	11.64	6.77	3.49
II 9 (11)	MD	25.96	12.69	8.05
	βCD	30.83	25.14	9.92
	αCD	28.27	19.63	8.69
	–	29.61	20.39	0.13
I 21 (33)	MD	29.03	21.33	15.99
	βCD	30.27	21.40	21.00
	αCD	34.43	19.43	8.77
	–	40.01	15.12	9.89
II 7 (30)	MD	16.65	9.13	3.75
	βCD	20.02	11.81	11.01
	αCD	18.29	10.08	4.24
	–	16.88	8.67	3.85

[a] MD, maltodextrin; βCD, β-cyclodextrin; αCD, α-cyclodextrin; –, juice samples without dextrins.

Table III. Relative Anthocyanin Concentrations in the Pigment Mixtures Produced by XAD-2 Resin Stored with Dextrins as Solutions Expressed as the Sum of Actual Peak Areas

cherry selection	stabilizer[a]	weeks 0	2	12
Montmorency	MD	2.12	1.51	0.81
	βCD	2.52	1.42	1.64
	αCD	3.03	1.50	0.80
	–	2.51	1.14	1.01
English Morello	MD	12.62	7.97	4.42
	βCD	12.53	4.32	2.41
	αCD	12.28	7.79	5.33
	–	13.21	8.88	4.00
II 9 (11)	MD	44.15	28.49	6.55
	βCD	43.20	32.69	18.53
	αCD	38.31	32.61	4.01
	–	32.58	2.9	0.00

[a] MD, maltodextrin; βCD, β-cyclodextrin; αCD, α-cyclodextrin; –, samples without dextrins.

were also investigated with maltodextrin and α- and β-cyclodextrins. Cyclodextrins are water-soluble sugar oligomers that are capable of forming reversible complexes with molecules fitting into the cyclodextrin cavity (Brewster et al., 1989). Malto- and cyclodextrins are known to readily bind to molecules in any form, in contrast to the copigmentation effect which can only occurr in aqueous conditions (Brouillard et al., 1989). The juice samples and pigment isolates from tart cherry selections with α- and β-cyclodextrins and maltodextrin were stored and analyzed similarly to stabilization experiments with H_3PO_4 (Tables II–IV). The HPLC analysis of the samples at 12 weeks with βCD contained 49, 37, 32, 69, and 55% of the original anthocyanin levels, respectively, for Montmorency, English Morello, II 9 (11), I 21 (33), and II 7 (30). The stabilization of anthocyanins in the juice samples stored with αCD and MD, at 12 weeks, was found to be lower than that of βCD (Table II). The juice samples stored without any dextrin were found to contain lower levels of anthocyanins after 12 weeks of storage and 0% for

Table IV. Relative Anthocyanin Concentrations in the Pigment Mixtures Produced by XAD-2 Resin Stored with Dextrins as Dry Powders Expressed as the Sum of Actual Peak Areas

cherry selection	stabilizer[a]	weeks 0	2	12
Montmorency	MD	1.34	0.41	0.31
	βCD	1.50	0.67	0.52
	αCD	1.37	0.48	0.41
	-	0.81	0.65	0.25
English Morello	MD	6.21	3.91	3.22
	βCD	6.75	5.22	4.94
	αCD	5.73	3.91	3.78
	-	7.26	3.85	3.85
II 9 (11)	MD	18.23	16.99	16.57
	βCD	24.02	24.84	20.34
	αCD	18.91	18.13	16.20
	-	21.56	18.83	10.83

[a] MD, maltodextrin, βCD, β-cyclodextrin; αCD, α-cyclodextrin; -, samples without dextrins.

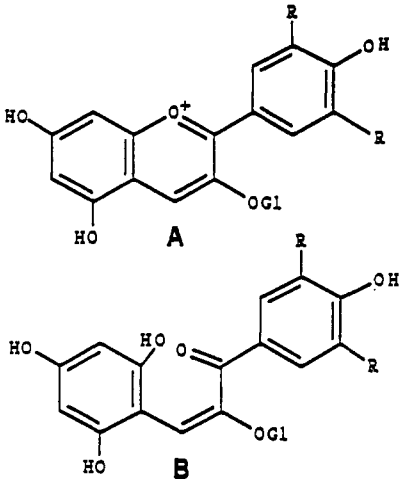

Figure 1. Structural forms of anthocyanins: (A) flavylium cation; (B) chalcone.

Montmorency under similar conditions. Except in Montmorency, which was stabilized by αCD, the rest of the juice samples were stabilized better with βCD.

The anthocyanin pigments isolated by using XAD-2 resin from Montmorency, English Morello, and II 9 (11) stored as aqueous solutions in association with βCD were found to contain higher levels of original anthocyanins than the samples stored with αCD and MD after 12 weeks (Table III). However, the aqueous solutions of the pigment mixtures stored devoid of dextrins were found to contain 40, 30, and 0% of the anthocyanins for Montmorency, English Morello, and II 9 (11), respectively.

Maintaining the flavylium cation A (Figure 1) is essential to retain the red color of anthocyanins in solution (Skrede, 1985; Van Buren et al., 1960). The colorless carbinol which is predominant at pH 4.5 accelerates the formation of chalcone B (Figure 1) and is responsible for anthocyanin degradation (Brouillard, 1982). Previous attempts of McLellan and Cash (1979) to stabilize the red anthocyanin color from Maraschino cherries by the addition of metal salts such as $SnCl_2$ failed to preserve the red color. The equilibrium of the anthocyanin pigments toward the flavylium cation was maintained by lowering the pH of the juice using H_3PO_4. The juice samples were stored under sterile conditions to avoid any microbial degradation.

The optimum activity of PPO was found to be in the pH range 5.6–6.3, suggesting the chalcone form to be responsible for the enzymatic degradation (Van Buren et al., 1960; Pifferi and Cultrera, 1974). However, degradation was still observed in all samples to some extent (Table I) and indicated that, besides the enzymes, there are other factors contributing to the degradation of anthocyanins. This observation is in agreement with the fact that Montmorency, II 7 (30), and II 9 (11) have similar PPO levels of 120–138.5 units/g (Iezzoni et al., 1991) but gave different degradation rates after 12 weeks (Table I).

The anthocyanin powders from Montmorency and English Morello stored as a complex with βCD in dry conditions were superior in stability than αCD and MD complexes under similar conditions (Table IV). However, MD complex with II 9 (11) was more stable than its complex with α- and βCD. The pigments from II 9 (11) gave higher anthocyanin levels during the storage without any stabilizer when compared to Montmorency and English Morello. All of the samples that were stored as powders with various dextrins were more stable than their aqueous solutions.

The aqueous solution of the dried pigments from XAD-2 resin was voilet in color at pH 6.1 and bright red at pH 3.5. The aqueous solution of the pigment isolate was heated in a microwave oven for 10 min at high energy. Also, the pigment isolate was separately heated on a water bath (100 °C) for 60 min. In both instances, the red color of the solutions persisted. However, an aqueous solution of the pigment heated in an oven at 230 °C for 40 min resulted in the total degradation of the anthocyanins.

We have used the XAD-2 resin with H_2O and EtOH as eluting solvents for the isolation of anthocyanins compared to the use of halogenated solvents reported earlier (Mirabel and Meiller, 1978). The pigments produced from tart cherries using XAD-2 resin can be stored at ambient conditions with very little degradation for more than 3 months. Complexing the flavylium cation form of the anthocyanin with dextrins prevented their transformation to other less stable forms. The pigment mixtures were found to be more stable as a powder than in solution.

ACKNOWLEDGMENT

This research is a contribution from the Michigan State University Agricultural Experimental Station and was partially funded by a grant from the Cherry Marketing Institute Inc. (CMI). We are thankful to Mr. C. R. Johnston, CMI, for supplying the juice concentrates of Montmorency and English Morello cultivars of tart cherries.

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Received for review January 12, 1993. Revised manuscript received March 25, 1993. Accepted April 14, 1993.