Changes in Anthocyanins and Polyphenolics During Juice Processing of Highbush Blueberries (*Vaccinium corymbosum* L.)

G. SKREDE, R.E. WROLSTAD, AND R.W. DURST

ABSTRACT: Frozen blueberries (*Vaccinium corymbosum* L.) were processed into juice and concentrate, and the changes in anthocyanin pigments and polyphenolics (cinnamates, procyanidins, flavonol glycosides) were monitored. While juice yield was 83%, only 32% of the anthocyanins were recovered in single-strength juice. Flavonol, procyanidin and chlorogenic acid recoveries in juice were 35%, 43%, and 53%, respectively. The proportion of polyphenolics remaining in the press-cake residue ranged from 1% (chlorogenic acid) to 18% (anthocyanins). Pronounced losses of anthocyanins and polyphenolics during milling and depectinization are believed to be due to native polyphenol oxidase. Losses during concentration ranged from 1.5% (anthocyanins) to 20% (procyanidins). Striking changes occurred in the anthocyanin profile with malvidin glycosides being most stable and delphinidin glycosides the least.

Key Words: anthocyanin pigments, polyphenolics, blueberry juice, processing, *Vaccinium corymbosum* L.

Introduction

INTEREST IN FRUIT COMPOSITION HAS INTENSIFIED BECAUSE OF increased awareness of the possible health benefits of some of their micronutrients (Rice-Evans and others 1996). Kalt and Dufour (1997) reviewed a number of the beneficial effects ascribed to both wild and domestic blueberries, including reduction of coronary heart disease, treatment of urinary tract disorders, and anticarcinogen activity. The anthocyanin pigments of the native European blueberry (or bilberry) *Vaccinium myrtillus* have long been used for improving visual acuity and treating circulatory disorders (Mazza and Miniati 1993). There are 184 pharmaceutical products derived from *V. myrtillus* (Kalt and McDonald 1996). Many of these biological properties are believed to be associated with the antioxidant activity of anthocyanin pigments, flavonoids, and other phenolic compounds (Rice-Evans and others 1996; Wang and others 1997). Prior and others (1998) measured the total anthocyanins, total phenolics, and oxygen radical absorbing capacity (ORAC) for 4 *Vaccinium* species and found a linear relationship between ORAC and anthocyanin and total phenolic content; they reported blueberries to be one of the richest sources of antioxidant phytonutrients of fresh fruits and vegetables studied.

The anthocyanin and polyphenolic composition of domestic highbush (*V. corymbosum* L.), lowbush (*V. angustifolium* Ait.), and European native blueberries (*V. myrtillus* L.) are qualitatively very similar (Francis and others 1966; Ballinger and others 1972; Sapers and others 1984; Azar and others 1987; Macheix and others 1990; Mazza and Miniati 1993; Gao and Mazza 1994; Kader and others 1996; Kalt and McDonald 1996). The anthocyanins are responsible for the bluish red color of the berries (Ballington and others 1987). Other flavonoids of importance are the flavonols, including the 3-*o*-glycosides of quercetin (Bilyk and Sapers 1986) and kaempferol (Kader and others 1996), catechin, epicatechin, and several procyanidins (Stöhr and Herrmann 1975). Chlorogenic acid is the most predominant cinnamic ester (Schuster and Herrmann 1985; Kader and others 1996, 1997b).

Blueberries are often processed into juice or juice concentrates for subsequent use in beverages, syrups, and other food products. Knowledge of the changes that the anthocyanin pigments undergo with processing is important with respect to their role in color quality. Anthocyanins as well as other polyphenolics are readily oxidized because of their antioxidant properties and, thus, susceptible to degradative reactions during various processing unit operations. Because of their possible beneficial roles as micronutrients, it is critical that changes in polyphenolics during processing be measured to better assess the dietary value of the processed products. Kader and others (1997a, 1997b) recently reported that native polyphenol oxidase in highbush blueberries accelerated anthocyanin pigment destruction in crushed blueberries. The degradative reactions were stimulated by the presence of chlorogenic acid. The present study was undertaken to investigate the changes in blueberry anthocyanins and polyphenolics during processing into juice and concentrate.

Results and Discussion

Mass balance and anthocyanin recovery during juice processing

The initial juice yield was 83% with the press-cake residue accounting for 10% of the starting material (Table 1). Thus, there was a 7% loss of material in the milling, enzyme maceration, and pressing unit operations. The total monomeric anthocyanin content in the blueberries used for juice processing was 99.9 mg/100 g of fresh berries, which conveniently allows for interconversion of calculated recoveries on either a mg/100 g or percentage basis. The pigment content is in good agreement with previously reported values of 111 mg anthocyanins/100g fresh weight (Gao and Mazza 1994) and 83.2 mg/100 g (Kalt and Dufour 1997) for the Bluecrop variety. The value falls within the wide range of 25 to 495 mg/100 g for highbush blueberries reported by Mazza and Miniati (1993).

The anthocyanin content of the initial pressed juice was 33.6 mg/100 g, while the press-cake residue contained 184 mg/100 g (Table 1). Anthocyanins are not as efficiently extracted in the pressing operation as are sugars, acids, and other water solubles. Thus, the press-cake is a potential pigment source for subsequent extraction, its total pigment content representing 18% of the anthocyanins in the starting material. The combined anthocyanin content of the initial pressed juice and press-cake residue

Table 1—Yield, °Brix, and total anthocyanin content during juice processing[a]

	Blueberry fruit	Initial pressed juice	Press-cake residue	Pasteurized juice	Concentrate
Yield (% w/w)	100	83	10	–	–
°Brix	15.1	15.0	–	15.0	73.5
Total Anthocyanin					
Sample (mg/100 g)	99.9	33.6	184	38.4	178
Blueberries (mg/100 g)	99.9a[b]	28.0d	18.2	32.0b	30.5c

[a]Anthocyanin content is reported for both the actual sample (row labeled Sample) and that derived from 100g of berries (row labeled Blueberries). Juice compositional data is reported on the basis of the actual °Brix, 15.0 °; to convert to the USA single-strength °Brix standard of 10.0 ° (Anon. 1993), multiply by 0.667.
[b]Numbers with different letters were significantly different ($p < 0.05$).

was 46.2 mg for 100 g of blueberries. If one assumes that the 7% material loss in processing had an equivalent pigment content, this would represent 46 + 7 or 53 mg/100g with an estimated loss of 47 mg anthocyanin/100 g fruit in processing operations. This low recovery of anthocyanin pigment clearly demonstrates extensive anthocyanin degradation under these processing conditions.

The anthocyanin content of pasteurized single-strength juice is actually significantly higher (4%) than that of the initial pressed juice. A plausible explanation is that pigments were destroyed in the initial pressed juice sample during freezing and thawing and/or storage by enzymes that were inactivated with pasteurization. Since the press-cake residue was not heat-treated, it is possible that it may actually be richer in anthocyanins than indicated by sample analysis if pigment destroying enzymes remained in this material. The anthocyanin content of the final pasteurized products was 38.7 mg/100 g for single-strength juice and 178 mg/100 g for concentrate. This represents a significant estimated pigment loss of 1.5% with concentration.

Enzymes responsible for anthocyanin degradation during juice processing could be endogenous enzymes in blueberry fruit, but an additional possible source could be side-activities of the commercial enzyme preparation used for depectinization. Supplemental experiments (data not shown) demonstrated that no glycosidase activity and no anthocyanin degradation activity was associated with the depectinization enzymes. In an additional experiment, crushed pulp from peeled blueberries (blanched and not-blanched) was added to pasteurized blueberry juice. Anthocyanin pigment content was monitored over 3 h at 40 °C. The results are shown in Fig. 1. Pasteurized juice lost about 50% of the anthocyanin content when incubated with crushed, anthocyanin-free, peeled blueberry pulp. The control containing blanched blueberry pulp showed no anthocyanin degradation. This clearly demonstrates that heat labile factors in blueberry pulp accelerate anthocyanin pigment destruction and strongly supports the hypothesis that endogenous enzymes in blueberry fruit cause pigment destruction in juice processing.

Kader and others (1997b) recently reported that endogenous polyphenol oxidase (PPO) from highbush blueberries caused anthocyanin degradation in crushed fresh blueberries. Chlorogenic acid also underwent severe degradation and its addition aggravated browning reactions and anthocyanin destruction. They concluded that PPO rather than peroxidase was responsible for the degradative reactions. Yokotsuka and Singleton (1997) have demonstrated that chlorogenic acid will stimulate PPO destruction of anthocyanins during grape juice processing. The chlorogenic acid content of blueberries (Table 4) is an additional compositional factor, which may be contributing to the pronounced color degradation observed in this investigation.

Blueberry anthocyanins

Figure 2A shows a typical high-performance liquid chromatogram (HPLC) of anthocyanins extracted from highbush blueberries of the Bluecrop variety. The anthocyanin isolate had been purified by acidic methanol elution from a C-18 Sep-Pak cartridge after the other polyphenolics had been eluted with ethyl acetate. Peak assignments were made on the basis of their ultraviolet (UV) -visible spectra and elution order as reported by Ballington and others (1987) and Gao and Mazza (1994), who used similar reverse-phase HPLC separation systems. The 12 peaks represent the 3-*o*-galactosides, glucosides and arabinosides of delphinidin, petunidin and malvidin, the 3-*o*-galactoside and glucoside of cyanidin, and 3-*o*-galactoside of peonidin. These identities were confirmed by electrospray mass spectroscopy (ESMS) (Fig. 2B, Table 2). The galactosides and glucosides of each anthocyanidin will give identical mass/charge ratios (m/z). In addition, a hexose cyanidin derivative and pentose petunidin derivative differ by only 0.2 mass/charge units. The 12 peaks in the HPLC chromatogram are reduced to 6 peaks in the ESMS spectrum (Fig. 2B). There are 3 late-eluting peaks, which remain unidentified in the HPLC chromatogram. However, their retention time is consistent with their being the acetylated derivatives reported by Gao and Mazza (1994). Mass/charge units of 507 (peak 13) and 535 (peak 14) were detected (Fig. 2B) which correspond to the acetylated derivatives of delphinidin-galactoside/glucoside and malvidin-galactoside/glucoside, respectively. Additional identification criteria were obtained from HPLC of the anthocyanidins obtained from acid hydrolysis of the blueberry anthocyanin extract. Delphinidin (26%), cyanidin (11%), petunidin (17%), and malvidin (44%) are present in substantial amounts with peonidin detected in minor (2%) quantities (Fig. 3A). ESMS gave evidence (Fig. 3B) for presence of the following anthocyanidins: cyanidin (m/z 287), peonidin (m/z 301), delphinidin (m/z 303), petunidin (m/z 317) and malvidin (m/z 331).

Table 2—Peak assignments for highbush blueberry (var. Bluecrop) anthocyanins analyzed by HPLC and electrospray mass spectroscopy (ESMS)

Anthocyanin	HPLC[a] Peak #	ESMS Mass/charge ratio m/z
Delphinidin-3-galactoside	1	465.2
Delphinidin-3-glucoside	2	465.2
Cyanidin-3-galactoside	3	449.2
Delphinidin-3-arabinoside	4	435.0
Cyanidin-3-glucoside	5	449.2
Petunidin-3-galactoside	6	479.2
Petunidin-3-glucoside	7	479.2
Peonidin-3-galactoside	8	463.2
Petunidin-3-arabinoside	9	449.0
Malvidin-3-galactoside	10	493.2
Malvidin-3-glucoside	11	493.2
Malvidin-3-arabinoside	12	463.0
Acetylated delphinidin-galactoside/glucoside	13	507.0
Acetylated malvidin-galactoside/glucoside	14	535.0

[a]Peak assignments according to Ballington and others (1987) and Gao and Mazza (1994).

Changes in the anthocyanin profile with juice processing

Table 3 compares the proportions of the individual anthocyanins in blueberry fruit with initial pressed juice, press-cake residue, pasteurized single-strength juice, and juice concentrate.

Table 3—Distribution (%) of highbush blueberry (var. Bluecrop) anthocyanins during juice processing

Anthocyanin	Peak #	Blueberry fruit	Initial pressed juice	Press-cake residue	Pasteurized juice	Concentrate
Del-3-gal	1	12.3	0.5	7.8	5.0	4.8
Del-3-glu	2	5.4	0.4	4.2	4.2	4.3
Del-3-ara + Cy-3-gal	3,4	12.0	0.4	10.6	6.8	6.6
Cy-3-gluc[a]	5	–	–	–	–	–
Pet-3-gal	6	9.1	3.0	7.7	5.9	6.2
Pet-3-glu	7	7.2[b]	1.7	5.7	4.8	4.2
Peo-3-gal	8	–	3.4	4.4	2.7	3.5
Pet-3-ara	9	6.3	2.6	6.9	4.2	3.1
Mal-3-gal	10	20.2	33.4	21.9	25.2	27.0
Mal-3-glu	11	10.6	24.4	12.9	19.5	19.1
Mal-3-ara	12	13.5	21.8	17.5	17.9	16.8
Total (%)		96.6	91.5	99.6	96.3	95.6

[a]Concentration too low for quantitation.
[b]Likely to include both pet-3-glu and peo-3-gal.

Peaks 3 and 4 (cyanidin-3-galactoside and delphinidin-3-arabinoside) were insufficiently resolved for quantitation and are listed as one combined value. The low concentration of cyanidin-3-glucoside (peak 5) did not permit accurate quantitation. Pronounced changes occur during processing, with the most dramatic difference being between the berry fruit and the initial pressed juice. Obviously, the changes in the unpasteurized sample include changes induced by enzymatic activity subsequent to initial pressing, for example, during frozen storage and/or freezing and thawing of samples. The proportion of malvidin glycosides increases substantially through processing, malvidin derivatives comprising 44% of the total anthocyanins in blueberry fruit and increasing to 63% in pasteurized juice and concentrate, and to 80% for initial-pressed juice. (As discussed previously, enzyme activity during freezing, thawing, and/or storage must contribute to the reduced anthocyanin content of this unpasteurized sample). Delphinidin glycosides were the most unstable, delphinidin-3-glucoside decreasing from 12% in blueberry fruit to 5% in pasteurized juice and concentrate, and only being present in trace amounts in initial pressed juice. Substantial decreases also occurred with petunidin and cyanidin glycosides. Peonidin-3-galactoside was a minor pigment and showed little change. There was no apparent difference with respect to degradation rate and glycosidic substitution. This would support the proposition that glycosidase enzymes are not the cause of degradation since they tend to be very specific with respect to the glycoside structure (Wrolstad and others 1994). There were no noticeable changes in the anthocyanin profile when pasteurized juice was concentrated.

The relative anthocyanin stability is related to their chemical

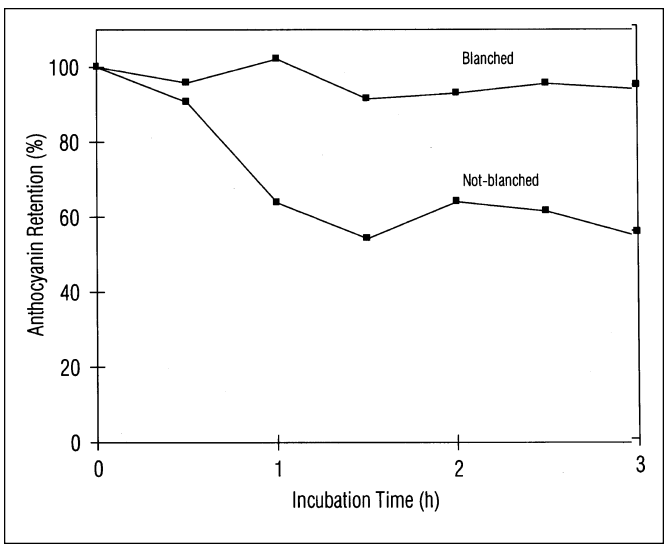

Fig. 1—Anthocyanin retention in pasteurized juice incubated with blanched and not-blanched blueberry (var. Bluecrop) pulp for 3 h at 40 °C.

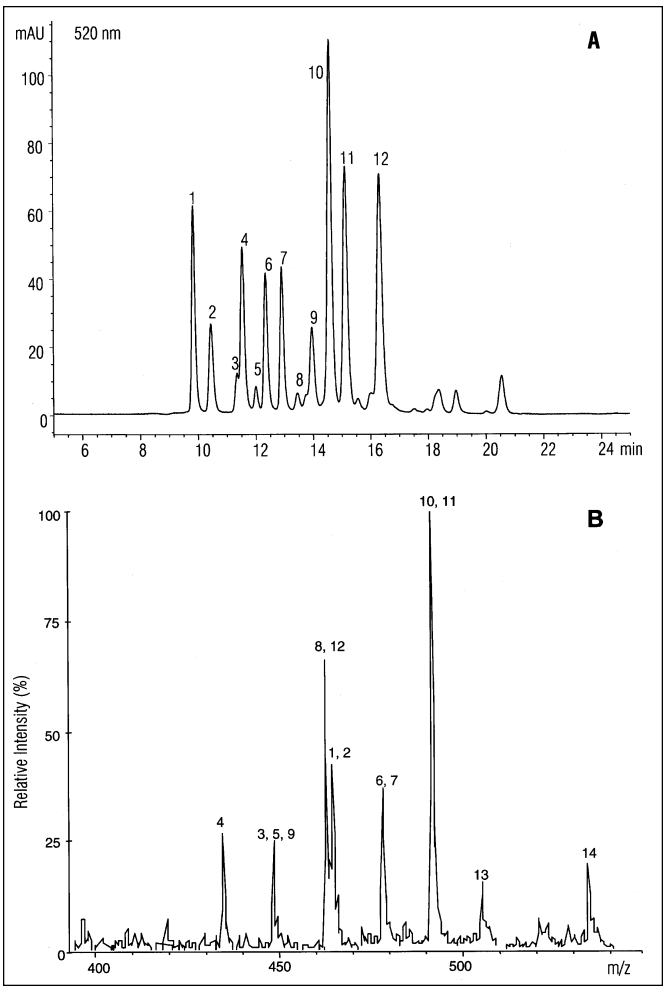

Fig. 2—HPLC chromatogram (A) and ESMS spectrum (B) of anthocyanins in highbush blueberry (var. Bluecrop) extracts. The anthocyanins were isolated by solid-phase extraction of C-18 cartridges with acidified methanol; cartridges had been previously washed with acidified water and by ethyl acetate. Peak identities for both the HPLC chromatogram and ESMS spectrum correspond to numbers in Table 2.

structure. The delphinidin glycosides with the greatest lability have 3 ortho phenolic groups in the B ring and the cyanidin and petunidin derivatives, which have the second order of reactivity, have 2 ortho phenolic groups. Peonidin and malvidin glycosides, which have the least reactivity, possess 1 phenolic substituent in the B ring with 1 and 2 adjacent methoxy substituents, respectively. Sarni and others (1995) showed that anthocyanins with ortho phenolic substitution in the B ring were oxidized via a coupled oxidation mechanism with *o*-quinones generated enzymatically from caffeoyltartaric acid. Anthocyanins with single phenolic substitution in the B ring reacted differently, forming adducts with the quinone; degradation of anthocyanins with ortho phenolic substitution occurred at a faster rate. Wang and others (1997) studied the ORAC of different anthocyanins and reported that cyanidin and delphinidin derivatives had higher Trolox equivalents than malvidin, peonidin, and pelargonidin derivatives. This order of ORAC activity is similar to the pigment stabilities observed in this study.

Changes in blueberry polyphenolics with juice processing

Chlorogenic acid. Chlorogenic acid is the major cinnamic acid derivative in highbush blueberries (Schuster and Herrmann 1985; Gao and Mazza 1994; Kader and others 1996). It was well resolved from other polyphenolics in the HPLC chromatogram (Fig. 4) and was identified by co-chromatography with an authentic standard and its matching UV-visible spectrum. The chlorogenic acid content of the blueberry fruit was 27.4 mg/100 g fresh weight (Table 4). This is much lower than the 185 mg/100 g reported by Schuster and Herrmann (1985) for Bluecrop berries, but similar to the 23 mg/100 g value reported by Kader and others (1997b) for the Colville variety.

Table 4 lists the content and recovery of polyphenolics with juice processing. In contrast to the anthocyanins, very little chlorogenic acid (1%) remains in the press-cake residue. Chlorogenic acid is more water-soluble and is found in the cell vacuole, whereas anthocyanins are less water-soluble and are associated with cell-wall material in epidermal tissue. Evidently chlorogenic acid is easily released with pressing. There was considerable loss of chlorogenic acid during processing, with about 50% of the chlorogenic acid present in the fruit remaining in the concentrate. There was about a 4% loss when pasteurized single strength juice was concentrated. The initial pressed juice contains less chlorogenic acid than the pasteurized juice, as was the case for the anthocyanins. Again, this indicates that enzymes inactivated with pasteurization were degrading chlorogenic acid during milling and pressing. Chlorogenic acid and caffeic acid have been shown to be good substrates for blueberry PPO (Kader and others 1997a), and the same authors have shown that extensive chlorogenic acid degradation occurs when fresh blueberries are homogenized (Kader and others 1997b).

Flavonol glycosides. Use of ethyl acetate in solid-phase extraction of C-18 resin cartridges effectively separated anthocyanins from other polyphenolics. Better HPLC resolution of polyphenolics was achieved when samples were prepared by that procedure. Peaks 8, 11, 12, 13, 14, and 15 (Fig. 5) had UV spectra characteristic of flavonol glycosides (Suárez Vallés and others 1994). Individual peak assignments were not made; however, Kader and others (1996) identified the main flavonol glycosides of highbush blueberries as quercetin-3-glucoside (isoquercitrin), quercetin-3-galactoside (hyperoside), and quercetin-3-rhamnoside (quercitrin); smaller amounts of kaempferol-3-glucoside were also reported to be present. In the present study, total flavonol glycosides calculated as rutin was 40.1 mg/100 g blueberry fruit (Table 4). This compares with 24 to 29 mg/100 g

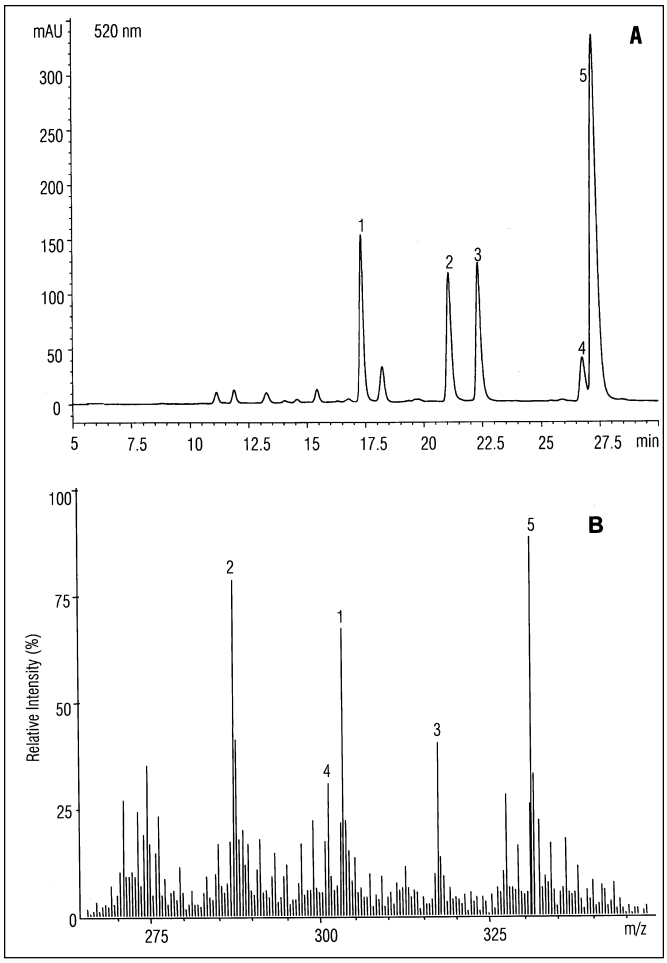

Fig. 3—HPLC chromatogram (A) and ESMS spectrum (B) of anthocyanidins after acid hydrolysis of purified anthocyanins from highbush blueberry (var. Bluecrop) extracts. Peak identification for both the HPLC chromatogram and ESMS spectrum: 1, delphinidin; 2, cyanidin; 3, petunidin; 4, peonidin; 5, malvidin.

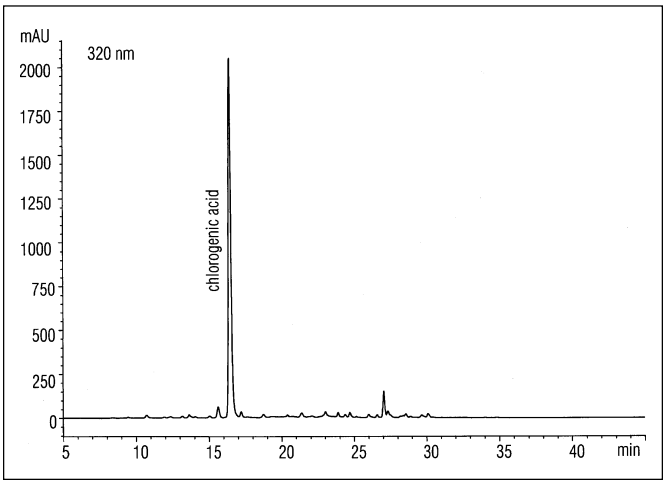

Fig. 4—HPLC chromatogram of polyphenolics in highbush blueberry (var. Bluecrop) extracts. The polyphenolics were isolated by solid-phase extraction of C-18 cartridges with acidified methanol; cartridges had been previously washed with acidified water.

Table 4—Content and recovery of chlorogenic acid, flavonol glycosides and procyanidins in highbush blueberries (var. Bluecrop) during juice processing[a]

	Blueberry fruit	Initial pressed juice	Press-cake residue	Pasteurized juice	Concentrate	Loss[b]
Chlorogenic acid						
Sample (mg/100 g)	27.4	13.2	3.2	17.4	79.8	1.8
Blueberries (mg/100 g)	27.4	11.0	0.3	14.6	13.7	1.8
Recovery (%)	100	40.2	1.2	53.3	49.9	6.7
Flavonol glycosides[c]						
Sample (mg/100 g)	40.1	24.9	26.8	16.7	71.7	2.7
Blueberries (mg/100 g)	40.1	20.8	2.7	14.0	12.3	2.7
Recovery (%)	100	52.8	6.6	35.0	30.6	6.7
Procyanidins						
Sample (mg/100 g)	9.9	4.5	2.8	5.0	13.4	0.7
Blueberries (mg/100 g)	9.9	3.8	0.3	4.2	2.3	0.7
Recovery (%)	100	38.0	2.8	42.6	23.0	6.7

[a]Polyphenolic content is reported for both the actual sample (row labeled Sample) and that derived from 100g of berries (row labeled Blueberries). Juice compositional data is reported on the basis of the actual °Brix, 15.0°; to convert data to the USA single-strength °Brix standard of 10.0° (Anon. 1993), multiply by 0.667.
[b]Estimated from 7% loss in yield during processing.
[c]Calculated as rutin.

fresh weight reported by Bilyk and Sapers (1986).

Considerable losses of flavonol glycosides occur with processing; however, the pattern is somewhat different than that for anthocyanins and chlorogenic acid. There is substantial loss with milling and pressing. Some flavonols remain in the press-cake residue, but not proportionally as much as the anthocyanins, that is, 7% compared to 18%. In contrast to anthocyanins and chlorogenic acid, the initial pressed juice contains higher levels than pasteurized single-strength juice and concentrate. This implies that the flavonol glycosides were not as susceptible to enzymatic degradation as the anthocyanins and chlorogenic acid. There was about a 5% loss of flavonol glycosides with concentration.

Procyanidins. A procyanidin fraction relatively free of anthocyanins and cinnamates was achieved using Sephadex LH-20 mini-column chromatography as described by Spanos and Wrolstad (1990). This sample preparation procedure was critical since a number of polyphenolics will co-elute with the 280 nm absorbing procyanidins. Fig. 5A shows the HPLC separation of compounds characterized as procyanidins by their UV spectra, that is, high 280/320 nm absorbance ratio. Catechin (peak 2) and epicatechin (peak 6) were identified by co-chromatography with authentic standards and matching UV spectra. Catechin's concentration in blueberry fruit was determined to be 1.6 mg/100 g. Stöhr and Herrmann (1975) reported that cultivated blueberries contain 0.9 to 2.0 mg catechin /100 g fresh weight and 1.1 to 1.5 mg epicatechin /100 g fresh weight. The procyanidin isolate from Sephadex LH-20 chromatography was hydrolyzed in 0.01 M HCl for 30 min; HPLC of the hydrolysate revealed decreases in procyanidins (peak 1 disappeared) with simultaneous increases in catechin and epicatechin (data not shown). This demonstrated that the procyanidins contained catechin and/or epicatechin subunits. Catechin, epicatechin, and procyanidin peaks were measured as catechin, their sum being nearly 10 mg/100 g in blueberry fruit (Table 4).

Extensive decreases in procyanidin levels occurred during processing, the amounts in initial pressed juice and single-strength pasteurized juice being about 40% of that in the fruit. Considerable degradation occurred with an approximate additional 20% loss, when single-strength pasteurized juice was concentrated. Relatively little (3%) of the procyanidins measured by HPLC remained in the press-cake residue. The changes in catechin and epicatechin during processing could not be monitored, as the levels were too low for accurate measurement.

Conclusions

THIS STUDY REVEALED THAT SUBSTANTIAL LOSSES OF ANTHOCYAnins and polyphenolics occurred when blueberries were processed into juice and concentrate and that different classes of compounds had varying susceptibility to degradation with different unit operations. Heavy losses of anthocyanins and chloro-

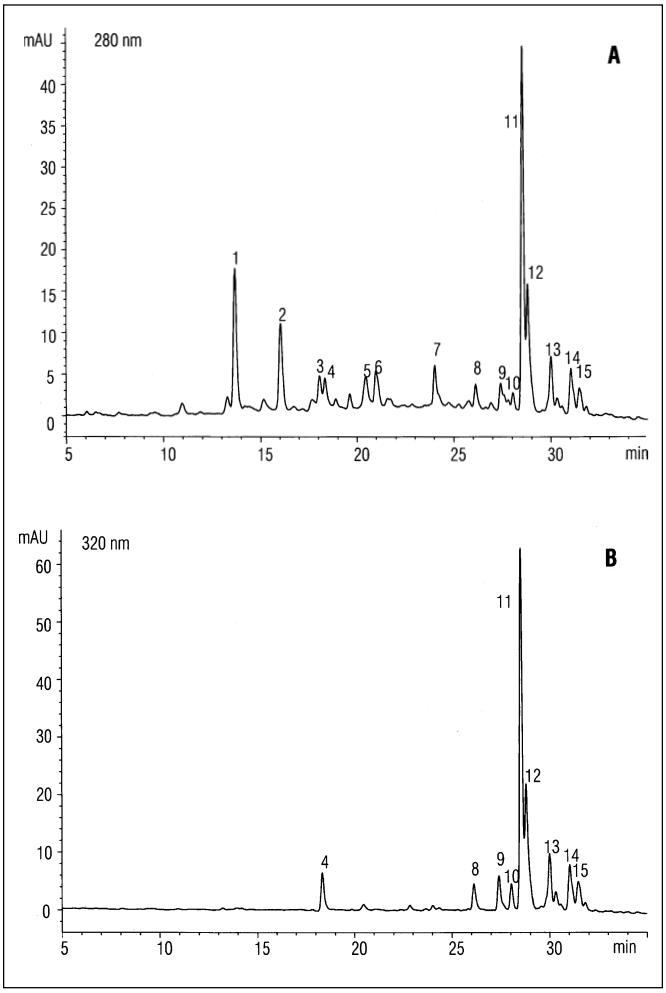

Fig. 5—HPLC chromatogram (A @ 280 nm, B @ 320 nm) of flavonol glycosides, procyanidins and catechin/epicatechin in highbush blueberry (var. Bluecrop) extracts purified by methanol elution from Sephadex LH-20 columns previously washed with 20% (v/v) methanol in water. Peak identification for both A and B: 2, catechin; 4, chlorogenic acid; 6, epicatechin; 1, 3, 5, unidentified procyanidins; 8, 11, 12, 13, 14, 15, unidentified flavonol glycosides; 9, 10, unidentified cinnamates; 7 unidentified.

genic acid occurred with milling and depectinization, which is believed to have been aggravated by native PPO. The anthocyanin profile changed drastically because of varying stability of individual pigments. This needs to be taken into consideration when evaluating processed blueberry products for authenticity. Future work needs to be directed to altering processing conditions to inactivate PPO and increase anthocyanin recovery. Considerable anthocyanins remained in the press-cake, which may be a potential colorant source. Polyphenolic and anthocyanin losses were relatively low when pasteurized single-strength juice was concentrated, except for the procyanidins, which showed marked reduction. These findings need to be taken into consideration when evaluating processed blueberry products for their possible health benefits.

Material and Methods

Fruit

For preliminary experiments at Oregon State University (OSU), individually quick frozen (IQF) highbush blueberries (*Vaccinium corymbosum* L., var. Bluecrop) grown at the North Willamette Experiment Station (Aurora, Oreg., U.S.A.) were used. For pilot-plant processing, commercially packed IQF berries were provided by the Oregon Blueberry Commission. The variety was unknown, but likely to be Bluecrop because of its appearance and predominance as a commercial crop in the growing region. Experiments on enzymatic destruction of pigments in frozen berries utilized Bluecrop variety grown at the Agricultural University of Norway (Ås, Norway).

Reagents and standards

Rapidase Super BE depectinization enzyme was obtained from Gist-Brocades International B.V. (Charlotte, N.C., U.S.A.). Phenolic standards (chlorogenic acid, catechin, epicatechin, and rutin) were obtained from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Aqueous stock solutions (400 mg/L) were prepared with addition of a few drops of ethanol to facilitate solution. Concord grape juice used for preparation of anthocyanidin standards was provided by Seneca Foods Corporation (Prosser, Wash., U.S.A.). All solvents used in this investigation were high-performance liquid chromatography (HPLC) grade.

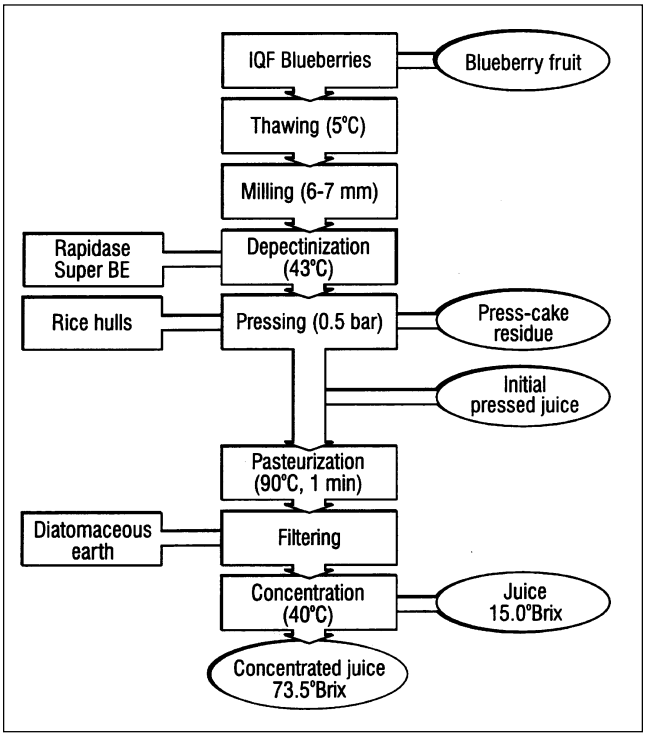

Fig. 6—Flow sheet for the processing of highbush blueberry (var. Bluecrop) fruit.

Juice Processing

Blueberries were processed into juice at the OSU Department of Food Science and Technology pilot plant according to the flow sheet shown in Fig. 6. The fruit (26 kg) was partially thawed (5 °C) and milled in a Fitzpatrick Model D Comminuting Machine (W.J. Fitzpatrick Co., Chicago, Ill., U.S.A.), using screen # 4, holes 6 to 7 mm. The crushed berries were subsequently heated to 43 °C in a hot water jacketed kettle equipped with a stirrer. Four mL of Rapidase Super BE depectinization enzyme preparation (initially diluted 1:10 with water) was added, followed by an additional 4 mL after 1.5 h. Depectinization was complete as indicated by a negative alcohol precipitation test after 2 h. Rice hulls (500 g) were added, and the pulp was pressed in a Wilmes bag press (Type 60, Moffet Co., San Jose, Calif., U.S.A.), the maximum pressure applied being 5.0 bar. Juice yield was measured and samples of press-cake residue and initial pressed juice were frozen for later analyses. Pasteurization (90 °C for 1 min) was performed using an APV-Crepaco high temperature short-time (HTST) unit, type "junior," APV-Crepaco Inc. (Tonawanda, N.Y., U.S.A.). Total time elapsed from maceration through pasteurization was approximately 4 h. Diatomaceous earth (150 g) was added, and the mixture was filtered through a filter unit (Hermann Strassburger, KG, Westhofen bei Worms, Germany), equipped with 9 filter pads (Seitz 20SQ, K-2600, 100 ct, Theo and Geo Seitz GmbH und Co., Bad Kreuznach, Germany). Residual rinse water in the pasteurizer and filter unit diluted the juice, which was restored to its original 15 °Brix using a Centritherm evaporator (Model CT-1B, Alfa-Laval Inc., Newburyport, Mass., U.S.A.), operated at temperature of 45 to 50 °C and vacuum of -0.85 to -0.9 kg cm^{-2}. A portion of the juice was further concentrated to 73.5 °Brix. Juice and concentrate samples were stored at -30 °C for subsequent analyses.

Blueberry pulp destruction of anthocyanins in pasteurized blueberry juice

Frozen blueberries (Bluecrop variety) were manually peeled with a scalpel while still frozen, the skins containing the anthocyanin pigments being discarded. The peeled fruit was crushed with a portion being blanched at 100 °C for 2 min. Four g of crushed blueberry pulp (blanched and not-blanched) were added to 4 mL pasteurized juice and 8 mL distilled water. Total anthocyanin content was monitored over 3 h at 40 °C.

Isolation of anthocyanins and polyphenolics from berries and press-cake residue

Anthocyanins and polyphenolics were extracted from blueberries and press-cake residue following the procedures described by Giusti and Wrolstad (1996). Frozen material was liquid nitrogen powdered using a stainless steel Waring blender.

Powdered samples (50 g) were blended with 100 mL acetone and filtered on a Buchner funnel using Whatman #1 paper. The residue was re-extracted twice with 100 mL acetone/water (70/30). Filtrates were combined, shaken in a separatory funnel with 2 volumes of chloroform and stored at 3 °C over night. The upper aqueous layer was collected and residual acetone removed using a Buchi rotary evaporator at 40 °C. The extract was made up to 100 mL with water containing 0.01% HCl; therefore, the extracts represented 0.5 g blueberries/press-cake residue/mL.

Isolation of anthocyanins and polyphenolics with C-18 resin

Juice concentrates were diluted to single-strength before treatment. Aqueous samples (1 mL) were applied to a C-18 Sep-Pak cartridge (Waters Assoc., Milford, Mass., U.S.A.), previously activated with 5 mL acidified methanol (0.01% HCl) followed by 2 mL acidified (0.01% HCl) water (Giusti and Wrolstad 1996). Sugars, acids, and other water-soluble compounds were eluted with 2 mL of 0.01% aqueous HCl and anthocyanins and polyphenolics were recovered with 2 mL acidified methanol. The methanolic extract was concentrated using a Buchi rotary evaporator at 35 °C, and taken up in 1 ml either acidified methanol or water dependent on the subsequent analysis.

An alternate procedure modified from the method described by Oszmianski and Lee (1990) and Auw and others (1996) yielded separate anthocyanin and polyphenolic isolates. The aqueous sample (1 mL) was applied to a C-18 Sep-Pak cartridge, which had been previously activated with successive applications of 5 mL ethyl acetate, 5 mL acidified (0.01% HCl) methanol, and 5 ml acidified (0.01% HCl) water. The cartridge with the absorbed sample was then washed with acidified water (5 mL), after which the cartridge was dried with a current of nitrogen for 3 min. Ethyl acetate (5 mL) eluted polyphenolics other than anthocyanins. The ethyl acetate eluant was evaporated to near dryness on a rotary evaporator (35 °C), taken up in acidified methanol and evaporated again. The sample was taken up in acidified water for subsequent analyses. Analysis indicated that 3% of the original anthocyanins were present in this fraction. The remaining anthocyanins were recovered from the cartridge with acidified methanol as described above.

Isolation of procyanidins with Sephadex LH-20

A procyanidin/catechin/epicatechin fraction was isolated using Sephadex LH-20 (Pharmacia, Uppsala, Sweden) mini-columns as described by Spanos and Wrolstad (1990). Sephadex LH-20 was swollen in water overnight and slurry packed in disposable Poly-Prep chromatography columns (0.8 × 4 cm graduated column with an integral 10 mL reservoir, Bio-Rad laboratories, Richmond, Calif., U.S.A.). Approximately 2 mL of chromatographic material filled the column. The polymer bed was rinsed with 5 mL distilled water and 2 mL aqueous sample was carefully applied. The bed was washed with 30 mL of 20% methanol in water, a slight vacuum being applied to assist percolation through the column; this fraction was discarded. The procyanidins were subsequently eluted with 15 mL methanol. The eluate was evaporated to dryness on a rotary evaporator (35 °C) and dissolved in 2 mL distilled water.

Preparation of anthocyanidins

Anthocyanin solutions (1 mL) were combined with 15 mL 2 N HCl in a screw-cap test tube, flushed with nitrogen, capped, and heated in a boiling water bath for 30 min (Hong and Wrolstad 1990). After cooling in an ice bath, the hydrolysate was applied to a C-18 Sep-Pak cartridge and the anthocyanidins recovered with acidified (0.01% HCl) methanol as described for isolation of anthocyanins above.

Total anthocyanin pigment content

Total monomeric anthocyanins were determined by the pH-differential method as described by Wrolstad and others (1982). Samples were diluted with buffers directly in disposable cells (1 cm path length) and the absorbance read at 520 nm on a Shimadzu 300 UV-Visible spectrophotometer. Pigment content was calculated as cyanidin-3-glucoside using extinction coefficient (E 1 cm 1% = 26,900) and molecular weight of 445 (Wrolstad, 1976).

°Brix

°Brix of juice was determined using an Auto Abbe refractometer model 10500 (Reichert-Jung, Leica Inc., N.Y., U.S.A.) with the temperature compensate mode.

HPLC analytical system

An analytical HPLC Perkin-Elmer Series 400 equipped with a Hewlett-Packard 1040A photodiode array detector and Gateway 2000 P5-90 computer with a Hewlett-Packard HPLC^{2D} ChemStation software was used. Sample injections (50 μL) were made via a Beckman 501 autosampler. Peaks were monitored by their absorbances at 280, 320, and 520 nm and quantified by their integrated peak areas using external standards. Absorbance spectra between 250 and 600 nm were recorded for all peaks. Solvents (HPLC-grade) and samples were filtered through a 0.45 μm Millipore filter type HA (Millipore Corp., Bedford, Mass., U.S.A.). The flow rate for all HPLC analyses was 1 mL/min and temperature was ambient. Peak assignments were made by co-chromatography of authentic standards (when available) and matching ultra-violet-visible spectra.

HPLC separation of anthocyanins

Anthocyanin separation utilized a Poly LC LC-18 column (5 μm) 250 × 4.6 mm i.d. (Poly LC Inc., Columbia, Md., U.S.A.), fitted with an Allsphere 10 × 4.6 mm i.d. ODS-2 guard column (Alltech, Deerfield, Ill., U.S.A.). Solvent A: 100% acetonitrile; B: 1% phosphoric acid, 10% acetic acid, 5% acetonitrile (v:v:v) in water. The program followed a linear gradient from 2 to 12% A in 15 min, from 12 to 22% A in 5 min and isocratic conditions with 22% A for 5 min.

HPLC of anthocyanidins

Anthocyanidins were separated using a Poly LC LC-18 column (5 μm) 250 × 4.6 mm i.d. fitted with an Allsphere 10 × 4.6 mm i.d. ODS-2 guard column (Alltech, Deerfield, Ill., U.S.A.). Solvents A and B were as described for anthocyanins above. A 30 min linear gradient from 0 to 30% A was used.

HPLC separation of polyphenolics

Flavonol glycosides, procyanidins, and cinnamates were separated on a Supelcosil (5 μm) 250 × 4.6 mm i.d. analytical column (Supelco Inc., Bellefonte, Pa., U.S.A.) equipped with a Spherisorb ODS-10, 40 × 4.5 mm i.d. micro-guard column (Alltech, Deerfield, Ill., U.S.A.). Solvent A: 100% acetonitrile; B: 100% methanol; and C: 0.07M KH_2PO_4 adjusted to pH 2.4 with

conc. H_3PO_4. Initial solvent composition was 10% B and 90% C, followed by a linear gradient from 10 to 22% B, 90 to 78% C in 10 min; a linear gradient with A from 0 to 25%, B constant at 22% and C from 78 to 53% in 25 min and a final isocratic period of 10 min. Flavonols were quantitated as rutin by the external standard method, procyanidins, catechin and epicatechin as catechin, and cinnamates as chlorogenic acid.

Electrospray mass spectroscopy (ESMS) of anthocyanins and anthocyanidins

Low-resolution mass spectroscopy was performed using electrospray ionization. The instrument was a Perkin-Elmer SCI-EX API III+ mass spectrometer equipped with an Ion Spray source (ISV = 4700, Orfice voltage = 80). The instrument was operated in the positive ion mode. Samples were dissolved in distilled water or HPLC grade methanol containing 0.01% HCl and introduced into the ESMS flow stream (1:1 acetonitrile:water with 0.1% TFA) by loop injection (5 μL).

Statistical analysis

Effect of processing on anthocyanin content of juices was analyzed by one-way analysis of variance (Minitab v. 12; Minitab Inc., State College, Pa., U.S.A.). Significant differences ($p \leq 0.05$) between means were determined by Tukey's method.

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