Impact of Juice Processing on Blueberry Anthocyanins and Polyphenolics: Comparison of Two Pretreatments

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ABSTRACT: Frozen blueberries (*Vaccinium corymbosum* L. cv. Rubel) were pilot-plant-processed into juice and concentrate: 2 treatments (heat and SO_2) and a control. Pressed juice yield ranged from 74 to 83% (w/w), but only 13 to 23% of the anthocyanins and 36 to 39% of the polyphenolics were recovered in the pasteurized juice. A substantial amount of anthocyanins and polyphenolics (> 42% and > 15%, respectively of the starting material) were present in the presscakes. Measurements of total and individual flavonoids showed a great loss after the initial processing steps (thawing, crushing, and depectinization). Overall anthocyanin levels were higher in treated samples after each processing step, but polyphenolic levels remained similar to those in the control.

Keywords: anthocyanins, polyphenolics, processing, heat, processing, SO_2, *Vaccinium corymbosum* L.

Introduction

ANTHOCYANINS AND POLYPHENOLICS ARE secondary metabolites produced by the plant. They are a very diverse group of compounds (> 4000 flavonoids from plants have been identified). These compounds are important in the quality of food for their contribution to appearance, taste, and health benefits (Strack and Wray 1994).

There is intense interest in the possible health benefits of blueberries and blueberry products, because of their high antioxidant capacity, which is highly correlated to their anthocyanin and total phenolic content (Kalt and Dufour 1997; Prior and others 1998; Kalt and others 2000). While there have been numerous investigations in measuring the antioxidant capacity of different *Vaccinium* species (Wang and others 1996; Heinonen and others 1998; Prior and others 1998; Velioglu and others 1998; Kalt and others 1999b, 2000; Martín-Aragón and others 1999; Miller and others 2000; Smith and others 2000; Wang and Jiao 2000; Ehlenfeldt and Prior 2001), less attention has been given to compositional changes resulting from processing (Fuleki and Hope 1964; Van Teeling and others 1971; Amakura and others 2000; Skrede and others 2000).

Flavonoid degradation in blueberries and their processed products are serious problems. Along with the degradation of important micronutrients, color loss is also a significant concern. Studies indicate that native enzyme polyphenol oxidase (PPO, which is mainly located in the cytoplasm), anthocyanins, and polyphenolics (predominantly located in the vacuole) undergo significant degradation when the fruit is processed. Researchers have proposed that native blueberry PPO oxidizes polyphenolics to quinones, which subsequently react with anthocyanins to produce brown pigments (Kader and others 1997, 1998, 1999; Jiménez and García-Carmona 1999).

Heating has been shown to inhibit native enzyme activity (Kader and others 1997; Skrede and others 2000). The addition of sulfur dioxide (SO_2) has also been effective in inhibiting the activity of PPO (Montgomery and others 1982; Sayavedra-Soto and Montgomery 1986; Sapers 1993). Bakker and others (1998) have shown an increase in anthocyanin extraction when making red table wine by increasing the amount of SO_2 added.

Previous work done by Skrede and others (2000) demonstrated the pronounced deterioration of phenolic compounds in highbush blueberries when frozen blueberries were processed into juice and concentrate. The objective of this study was to determine the effectiveness of initial heat and sulfur dioxide treatments, to improve blueberry juice color quality. Anthocyanin and polyphenolic substances were measured from samples taken after each step in the processing of berries into pasteurized juice and, finally, into concentrate.

Materials and Methods

Plant material

Flow frozen highbush blueberries (*Vaccinium corymbosum* L. cv. Rubel) grown by Steve Erickson Farms (Salem, Oreg., U.S.A.) were provided by the Oregon Blueberry Commission. The frozen berries were stored at -23 °C until juice processing. Rubel is 1 of 3 wild highbush blueberries native to North America that have contributed to present-day cultivars (Hancock and Siefker 1982).

Reagents and standards

Cyanidin-3-glucoside and quercetin-3-glucoside were obtained from Extrasynthèse (Genay, France). Potassium metabisulfite, caffeic acid, ferulic acid, o-coumaric acid, rutin (quercetin-3-rutinoside), chlorogenic acid, vanillic acid, syringic acid, and p-hydroxybenzoic acid were obtained from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). The pectinase (Pectinex Smash Batch 1284210L) used in the juice processing was from Novo Nordisk Ferment Ltd (Dittingen, Switzerland). All solvents used in this investigation were HPLC grade.

Juice processing

The procedure for blueberry juice and concentrate processing is shown in Figure 1. Blueberries were processed into juice at the Oregon State Univ. Dept. of Food Science and Technology pilot plant. There were 3 groups: initial heat treatment, SO_2 treatment, and no treatment (control). Control fruits were thawed, crushed, depectinized, pressed, clarified, pasteurized, and concentrated. The heat treatment procedure differed only in that the

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thawed blueberries were immediately heated to 95 °C for 2 min, and then cooled to 38 °C. SO_2 treatment differed from the control by the addition of potassium metabisulfite ($K_2S_2O_5$) during thawing and crushing (free SO_2 = 100 ppm). All 3 trials were replicated 2 times. Approximately 20 kg of frozen blueberries were used for each trial. Frozen blueberries were thawed in a steam kettle equipped with stirrer until room temperature was reached. Thawed berries were crushed with a Stephan Vertical Cutter Mixer (Stephan Machinery Co., Cloumbus, OH, U.S.A.) set at 30 s and slow speed. Pectinase (0.0827 mL/kg) was then added, as it is most active at 27 °C. Negative alcohol precipitation test was used as an indication of depectinization. After depectinization, rice hulls (1%) were added to aid in juice pressing. Then the depectinized crushed berries were pressed in a Willmes bag press (Type 60; Moffet Co., San Jose, Calif., U.S.A.). The maximum pressure applied was 5.0 bar. Pressed juice was clarified using a continuous centrifuge (model KA2 separator; Westfalia, San Leandro, Calif., U.S.A.). Due to the small amount of pressed juice, a filter unit was not used. Clarified juice was pasteurized (90 °C for 90 s) with an APV-Crepaco high temperature short-time (HTST) unit, type "junior", APV-Crepaco Inc. (Tonawanda, N.Y., U.S.A.). Pasteurized juice was concentrated by a Centri-Therm centrifugal evaporator (model CT-1B; Alfa-Laval Inc., Newburyport, Mass., U.S.A.) operating at 40 °C. Pasteurized juice samples were concentrated to 65 to 70 °Brix. °Brix measurements were made with an Auto Abbe refractometer model 10500 (Reichert-Jung, Leica Inc., Buffalo, N.Y., U.S.A.) in % solids and temperature-controlled mode. Samples were taken from each step of juice processing after crushing for analysis. Samples taken were stored at -23 °C until analysis.

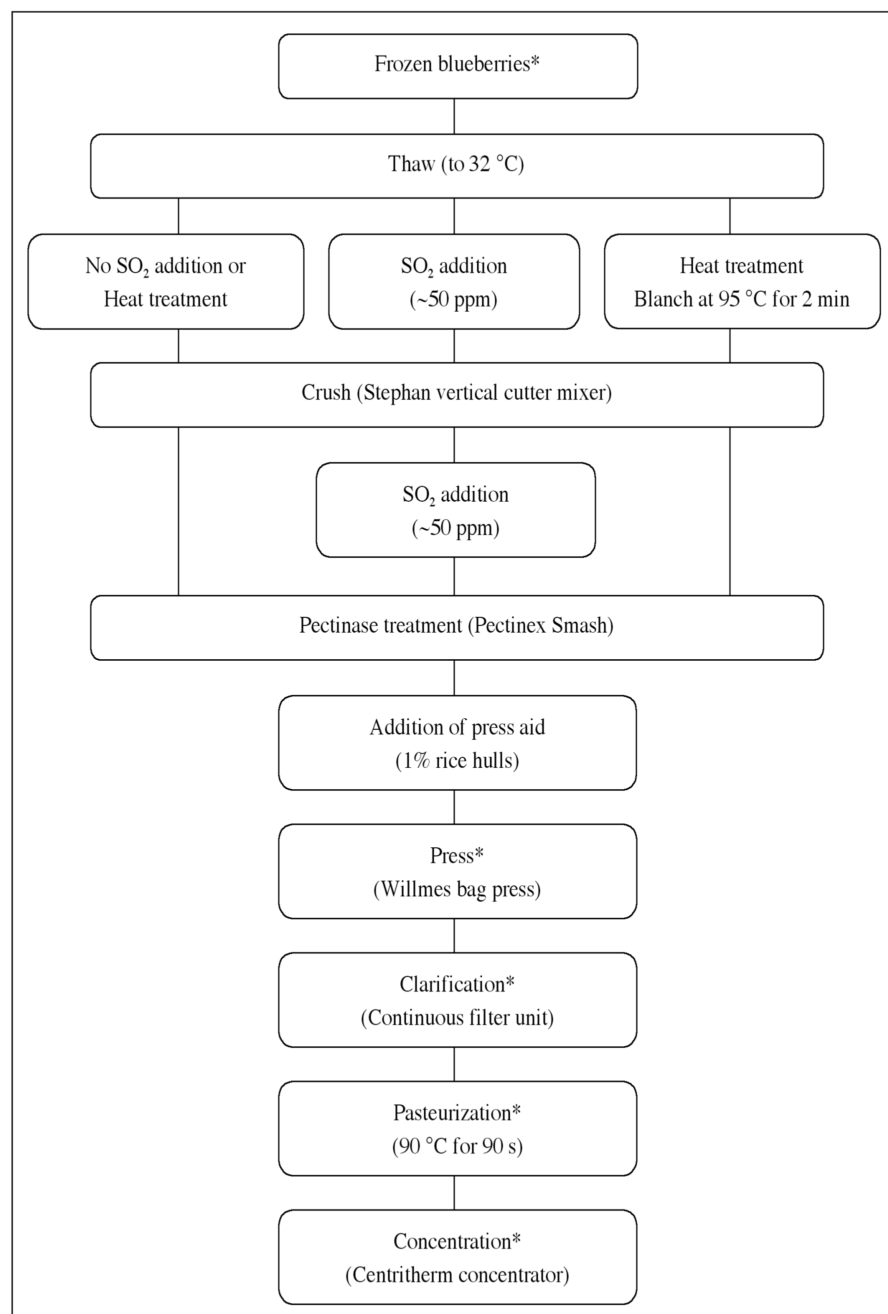

Figure 1—Blueberry juice and concentrate processing. (* samples taken for analysis)

Pigment extraction

Frozen blueberry and presscake sample extractions were performed following the procedure described by Rodriguez-Saona and Wrolstad (2001). Samples were liquid nitrogen powdered using a stainless steel Waring Blender. Twenty-five g of powdered sample was blended with 50 mL of acetone and filtered with a Buchner funnel and Whatman nr 1 filter paper. The filter cake was re-extracted with 70% acetone until a clear filtrate was obtained. Filtrates were combined in a separatory funnel with chloroform (1:2 acetone: chloroform v/v) and stored overnight at 1 °C. The aqueous portion was collected, and residual acetone was evaporated by a Büchi rotovapor at 40 °C. The extract was dissolved to a final volume of 150 mL with distilled water. The samples for anthocyanin quantification by high-performance liquid chromatography (HPLC) were filtered through a 0.45-µm Millipore filter (Type HA for HPLC samples) prior HPLC injection. The extractions for the berries and presscakes were replicated 2 times.

Total monomeric anthocyanin and % polymeric color content of samples

Total monomeric anthocyanin pigment (ACN) contents of berries, pasteurized juice, and concentrate were determined by using the pH differential method (Giusti and Wrolstad 2001). Absorbance was measured at 510 and 700 nm. ACN was calculated using cyanidin-3-glucoside coefficients (molar extinction coefficient of 26900 L cm^{-1} mol^{-1} and molecular weight of 449.2 g mol^{-1}).

Percent polymeric color contents of these samples were determined by the bisulfite bleaching method (Giusti and Wrolstad 2001). Absorbance was measured at 420, 510, and 700 nm. A Shimadzu 300 UV-visible spectrophotometer (Shimadzu Inc., Kyoto, Japan) and a 1-cm-pathlength cell were used for both measurements. Determination of ACN and % polymeric color were replicated 2 times.

Anthocyanin and polyphenolic sample preparation

Anthocyanin and polyphenolic purification were performed as described in Giusti and Wrolstad (1996). The aqueous extract was passed through a C-18 Sep-Pak mini-column (Waters Associates, Milford, Mass., U.S.A.), rinsed with methanol, and activated with deionized water.

Anthocyanins.

Anthocyanins were absorbed onto the column while other flavonoids, sugars, and acids were removed. Anthocyanins were collected with acidified (0.01% HCl) methanol, and samples for ES-MS analysis were then filtered through a 0.45-mm Millipore filter (type HV) before injection. For HPLC samples, the methanol was evaporated using a Büchi rotovapor at 40 °C. The pigments were re-dissolved in 2 mL acidified water (0.01% HCl) and filtered through a 0.45-mm Millipore filter (type HA) prior injection.

Polyphenolics.

The polyphenolic fraction was obtained by eluting 2 mL of ethyl acetate. Ethyl acetate was removed from the fraction with the Büchi rotovapor at 40 °C, then the residue was re-dissolved in 2 mL deionized water. Again, samples were filtered through a 0.45-µm Millipore filter (type HA) before HPLC injection.

Alkaline hydrolysis

Anthocyanins: The methanolic anthocyanin fraction was obtained as described previous. The methanol was evaporated and the fraction was redissolved in 10 mL of 10% KOH. Saponification was carried out in a screw-cap test tube at room temperature (23 °C) for 8 min. The solution was then neutralized with 2 N HCl. The hydrolysate was passed through a C-18 Sep-Pak mini-column and purified as described above for subsequent HPLC analysis.

Phenolics: Sugars and acids were removed using a C-18 Sep-Pak mini-column as described previous. Ethyl acetate was evaporated and the subsequent alkaline hydrolysis was performed as described for anthocyanins.

HPLC separation of anthocyanins and polyphenolics

Anthocyanins and polyphenolics were separated by reversed-phase HPLC using a Perkin-Elmer Series 400, equipped with a Hewlett-Packard 1040A photodiode array detector. Absorbance spectra were recorded for all peaks. Flow rate was 1 mL/min and injection volume was 50 µL.

Anthocyanins: A Prodigy 5 µm ODS (3) 100A (250 × 4.6 mm) column from Phenomenex was used (Torrance, Calif., U.S.A.). Solvent A was 100% acetonitrile and solvent B was 4% phosphoric acid. All solvents were HPLC grade. The program used a linear gradient from 6% to 25% solvent A, in 55 min with simultaneous detection at 280, 320, and 520 nm. Anthocyanins were quantified as cyanidin-3-glucoside by the external standard method (Durst and Wrolstad 2001).

Polyphenolics: A Supelcosil column (5 mm) 250 × 4.6 mm i.d. (Supelco Inc., Bellefonte, Pa., U.S.A.), fitted with a 10 × 4.6 mm i.d. Spherisorb ODS-2 micro-guard column (Alltech, Deerfield, Ill., U.S.A.) was used. Solvent A was 100% acetonitrile, solvent B was 100% methanol, and solvent C was 0.07 M KH_2PO_4 (adjusted to pH 2.4 with concentrated H_3PO_4). The initial solvent composition was 10% solvent B and 90% solvent C; then a linear gradient of 10% to 22% solvent B, and 90% to 78% solvent C in 10 min; then a linear gradient of solvent A from 0% to 25%, solvent B constant at 22%, and solvent C from 78% to 53% in 25 min; then isocratic for 10 min. Detection occurred at 260, 280, 320, and 520 nm. Flavonol-glycosides were quantified as rutin (quercetin-3-rutinoside) and cinnamates were quantified as chlorogenic acid by the external standard method.

Electrospray mass spectroscopy (ES-MS) of anthocyanins

Analysis was performed using a Perkin-Elmer SCIEX API III+ mass spectrometer, equipped with an ion spray source (ISV = 4700, orifice voltage = 80) and positive ion mode with loop injection. Purified and filtered anthocyanins in acidified methanol (5 µL) were injected directly into the system.

Determination of total sulfur dioxide (SO_2)

Total SO_2 concentrations were measured in juice and concentrate samples, after dilution to single-strength, by the Ripper method with a redox electrode (Zoecklin and others 1995). The redox electrode was from SensoreX (nr S500-ORP BNC, Stanton, Calif., U.S.A.) used with a Corning pH meter 320 (Corning, New York, N.Y., U.S.A.) in mV mode.

Color measurements

Measurements were made with a HunterLab CT1100 ColorQuest colorimeter (Hunter Associates Laboratories Inc., Reston, Va., U.S.A.). The colorimeter was set to measure total transmittance with Illuminant C and 10 ° observer angle. Pasteurized juice and concentrate samples were diluted to 10 °Brix (standard single-strength) with distilled water. An optical glass cell (Hellma, Borough Hall Station, N.Y., U.S.A.) with a pathlength of 0.25 cm was used to measure the samples. Three color parameters were recorded: Hunter CIE L* (lightness), chroma (saturation, C), and hue angle (color itself, h).

Statistical analysis

The significant difference among control, heat treatment and SO_2 treatment were determined at 95% level using the Tukey test of means. SAS statistical software (SAS systems for Windows, released 6.11, SAS Institute, Inc., Cary, N.C., U.S.A. 1996) was used.

Results and Discussion

Characterization of blueberry anthocyanins

The total monomeric anthocyanin content of Rubel blueberries was 192.4 mg/100 g determined by the pH differential method as cyanidin-3-glucoside (e = 26,900 and MW = 449.2). This was lower than the values of 235.4 and 220 mg/100 g reported for Rubel berries by Prior and others (1998, 2001), as well as 325 mg/100 g reported by Ehlenfeldt and Prior (2001).

The separation of anthocyanins by HPLC are shown in Figure 2. Peak assignments were made according to their ultraviolet (UV)-visible spectra and retention time. Electrospray mass spectroscopy (ES-MS) was used to confirm peak identification. There were 13 peaks in Rubel blueberries (in the HPLC elution order and the values in parentheses are the ES-MS mass/charge ratio) are as follows; delphinidin-3-galactoside (465.2), delphinidin-3-glucoside (465.2), cyanidin-3-galactoside (449.2), delphinidin-3-arabinoside (435.0), cyanidin-3-glucoside (449.2), petunidin-3-galactoside (479.2), cyanidin-3-arabinoside (419.0), petunidin-3-glucoside (479.2), peonidin-3-galactoside

(463.2), petunidin-3-arabinoside (449.0), malvidin-3-galactoside (493.2), malvidin-3-glucoside (493.2), and malvidin-3-arabinoside (463.0). There were no acylated anthocyanins (saponification did not eliminate peak 13, possibly a polymerized anthocyanin) detected by HPLC. Mass spectra data also showed no indication of acylated pigments, though Gao and Mazza (1994) reported the presence of acylated anthocyanins in highbush blueberries. Malvidin and delphinidin derivatives (77.2% of the constituents) were the major anthocyanins present in Rubel, which agrees with reported anthocyanin contents of *V. corymbosum* (Ballington and others 1987; Kader and others 1996; Kalt and others 1999a).

Changes in anthocyanin composition during juice processing

Table 1 shows the change in total and individual anthocyanidin-glycosides of each sample from the 3 juice and concentrate processing trials. The starting material (frozen blueberries) had 130.8 mg anthocyanins/100 g of berries (from HPLC), which agrees with previous reports. Gao and Mazza (1994) reported that highbush blueberries had an average of 110 to 260 mg of anthocyanins/100 g of fresh berries; and Mazza and Miniati (1993) reported a wide range of total anthocyanins (25 to 495 mg/100 g) for highbush blueberries. Pigment contents of the samples taken from the processing steps were expressed as mg of anthocyanins/100 g of initial frozen fruit.

Pressed juice

The primary steps of processing (thawing, crushing, depectinization, and pressing) contributed to a large loss in total anthocyanins, which was also reported by Skrede and others (2000). The crushed berries were visually brown. Pressed juice yields ranged from 75 to 83% (w/w). If no degradation occurred during juice processing, pressed juice samples would have in the order of 59.0 to 75.5 mg/100 g of the starting materials' anthocyanins, as 55.3 to 71.8 mg/100 g remained in the presscake. However, less than 22% (6.1 to 28.8 mg/100 g) of the frozen berries' anthocyanins were present in the pressed juices.

This low recovery of anthocyanins indicated degradation of the compounds. Control pressed juice had the lowest recovery, with significantly less (< 25%) anthocyanins than the heat-treated or SO_2-treated pressed juices ($p < 0.05$). Anthocyanins were not efficiently extracted into the pressed juice, which had a negative impact on juice quality.

Clarified juice

After clarification, heated and SO_2-treated juices had 4 times the anthocyanin content of control clarified juice ($p < 0.05$). There was approximately 8% loss of anthocyanins from pressed juices to clarified juices, less than 20% of the frozen fruit anthocyanins remained in any of the clarified juices.

Pasteurized juice. Heated and SO_2-treated pasteurized juices had 1.8 times the anthocyanin (29.8 and 30.4 mg/100 g, respectively) content of the control pasteurized juice (16.6 mg/100 g). There was greater than 76% loss of anthocyanins in the pasteurized juices compared to the frozen fruit. Control pasteurized juice had a higher amount of anthocyanins than the initial pressed juice. This may be due to enzymatic losses in the frozen samples during subsequent storage and thawing. Losses were observed with SO_2 and heat-treated samples, but not to the same extent as control samples. Twenty-five to 35% (32.4 to 45.7 mg/100 g) of the frozen berries' anthocyanins was degraded (or not accounted for in the final pasteurized juices and presscakes). Skrede and others (2000) demonstrated that this degradation is mainly due to native enzymes present in the blueberry fruit and not due to the processing enzymes used.

Concentrate

There was less than a 10% loss of anthocyanins from the concentration step. Anthocyanin contents of heat-treated and SO_2-treated (29.3 mg/100 g and 27.2 mg/100 g, respectively) concentrate samples were greater than the control concentrate (16.5 mg/100 g). However, the values were not significantly different ($p < 0.05$).

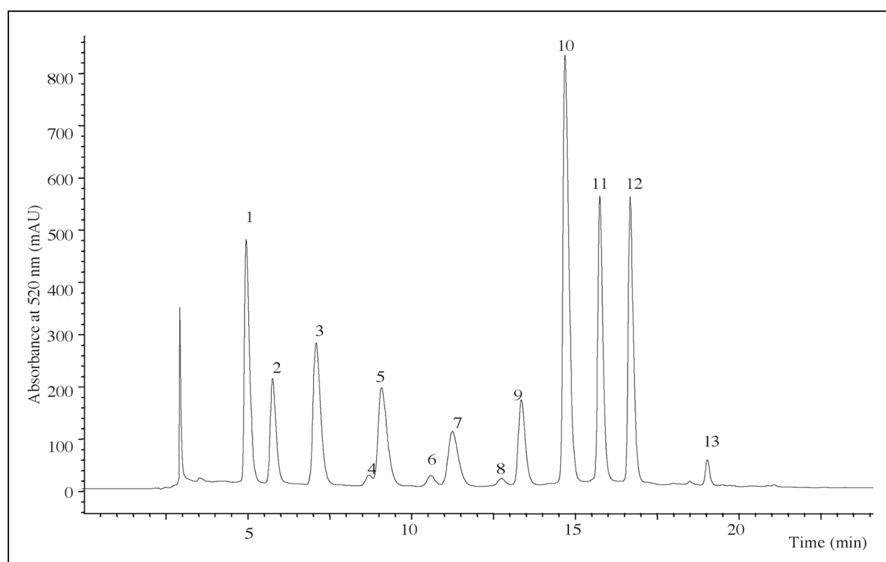

Figure 2—Anthocyanin HPLC profile of Rubel blueberries. 1: delphinidin-3-galactoside, 2: delphinidin-3-glucoside, 3: cyanidin-3-galactoside & delphinidin-3-arabinoside, 4: cyanidin-3-glucoside, 5: petunidin-3-galactoside, 6: cyanidin-3-arabinoside, 7: petunidin-3-glucoside, 8: peonidin-3-galactoside, 9: petunidin-3-arabinoside, 10: malvidin-3-galactoside, 11: malvidin-3-glucoside, 12: malvidin-3-arabinoside, and 13: unknown

Presscake

As mentioned earlier, the presscake residues contained a substantial amount of anthocyanins, 55.3 to 71.8 mg/100 g, or 42 to 55% of the frozen berries' anthocyanins. Control presscake contained the greatest amount of anthocyanins (71.8 mg/100 g), while heat treatment presscake had the least (55.3 mg/100 g). Heating the berries may have contributed towards a breakdown of the skins, which could have increased extraction of the anthocyanins, but still a substantial amount of anthocyanins remained in the presscake. The actual anthocyanin content of the presscakes could feasibly have been higher; any residual enzyme activity of the presscake may have degraded anthocyanins prior to analysis. The presscakes were not treated before storage to stop the activity of the native enzymes or the processing enzymes.

The presscake could be a source of anthocyanins for natural pigments and nutraceuticals (natural antioxidant source). Re-extracting the presscakes or crushing

Table 1—Total anthocyanin content in the different samples during the 3 juice processing trials (C: control, H: heat treatment, and S: SO_2 treatment)[1]

		Presscake			Pressed juice			Clarified juice			Pasteurized juice			Concentrate		
	Fruit	C	H	S	C	H	S	C	H	S	C	H	S	C	H	S
Delphinidin-glycosides	33.7	-	5.9	3.2	0.3	5.3	3.8	1.3	5.9	7.0	1.2	5.8	4.1	15.0	16.3	16.7
Cyanidin-glycosides	1.9	-	0.5	0.4	-	0.4	0.5	0.4	0.5	0.5	0.3	0.5	0.5	1.1	1.0	1.2
Petunidin-glycosides	25.7	0.2	4.9	3.4	0.1	4.5	3.8	1.9	5.0	4.1	1.9	5.0	4.1	13.7	11.3	14.5
Peonidin-glycosides	0.7	-	0.2	0.1	-	0.1	0.1	-	0.2	0.1	-	0.2	0.1	0.4	0.4	0.4
Malvidin-glycosides	67.3	5.6	16.9	17.2	5.5	15.7	17.6	12.7	17.9	18.2	12.8	17.4	17.9	40.6	25.8	34.4
Unknown	1.5	0.2	0.3	0.3	0.2	0.3	0.4	0.3	0.4	0.4	0.3	0.5	0.4	1.0	0.6	0.8
Total[2]	130.8	6.1[a]	28.8[b]	24.7[b]	6.1[a]	26.4[b]	26.2[b]	16.6[a]	29.8[a]	30.4[a]	16.5[a]	29.3[a]	27.2[a]	71.8[a]	55.3[a]	68.0[a]
SD	7.4	2.4	1.7	1.6	3.5	0.6	2.2	0.1	2.4	7.2	2.4	4.1	2.2	7.4	14.5	7.6

[1]:Units are mg of anthocyanin/100 g of berries (starting material).
[2]:Total with different lower case letters were significantly different ($p < 0.05$). To obtain single-strength 10 ºBrix of juice samples, multiply by 0.618. To obtain 65 °Brix for concentrate, multiply value by 4.02.

the frozen berries into fine particles may aid in higher extraction of anthocyanins into the final product. As presscake is mainly composed of fruit skins, which are high in pigment, blueberry varieties with greater anthocyanin content in their flesh (such as *V. membranaceum*) may yield higher levels of anthocyanins in their processed juices. The importance of anthocyanins remaining within the presscakes is quite significant, as Kalt and others (2000) contend that antioxidant capacity in blueberries is mainly due to anthocyanins, although other phenolics will also contribute to antioxidant activities. They also reported that the oxygen radical absorbing capacity (ORAC) can be predicted by simple colorimetric tests (pH differential test for anthocyanins and Folin-Ciocalteu test for phenolics).

Montgomery and others (1982) have reported that heating crushed Concord grapes helped increase anthocyanin extraction, and reduced polymeric color in the final juice samples. Studies performed by Kalt and others (2000) also indicate a high recovery of anthocyanins (and antioxidant capacity) in extracts of lowbush blueberry puréed at high temperatures (60 °C). They had a greater loss in these compounds, however, during storage at 20 °C, when compared to blueberries that were puréed at 25 °C.

Kader and others (1997) demonstrated that PPO activity plays a dominant role in enzymatic browning of blueberry anthocyanins, and that the addition of chlorogenic acid (major phenolic acid present in blueberries) stimulated the degradation of anthocyanins. Cyanidin-3-glucoside did not degrade in the presence of PPO alone, but by adding chlorogenic acid the anthocyanin deteriorated (Kader and others 1998). The addition of heat-inactivated PPO to the anthocyanins showed no degradation. Degradation of blueberry anthocyanins present in a juice system was shown by Skrede and others (2000) by the addition of blueberry pulp (endogenous enzymes present in the fruit would be present in the pulp).

Anthocyanin profile changes from processing

The proportion of malvidin-glycosides increased with the initial pressing compared to the fresh fruit. Malvidin-glycosides in the berry fruit were 51% of total anthocyanins and increased

to 60 to 77% in pasteurized juices and concentrates. There was a concomitant decrease in delphinidin and petunidin-glycosides. Skrede and others (2000) reported a considerable decrease in the proportion of delphinidin-glycosides; in this study that was true with the control (8%) but not with heat-treated (20%) and SO_2–treated (23%) pasteurized juices (fruit was 26%). Only 1.3 to 7.0 mg/100 g of delphinidin-glycosides remained in the pasteurized juice, compared to the fresh fruit delphinidin-glycosides content (33.7 mg/100 g). The proportion of cyanidin-glycosides increased in the pasteurized juice (control - 2.4%, heat - 1.7%, and SO_2 - 1.6%) and concentrate (control - 1.8%, heat - 1.7%, and SO_2 - 1.8%) compared to the fruit (1.5%). The anthocyanin profile changed only slightly during the concentration step.

Total monomeric anthocyanin, % polymeric color, and color measurements

Control-pasteurized juice and concentrate were higher in polymeric anthocyanins than heated and SO_2-treated juice and concentrates (Table 2). Total anthocyanin contents of pasteurized juices and concentrates obtained by pH differential were higher (1.5 to 1.9 times higher) than the results from HPLC, but provided the same general trend. The difference in results may be partially explained by anthocyanins spectral characteristics being influenced by the differing solvent systems utilized for HPLC and pH differential. Additionally, determination of total anthocyanins by HPLC was a summation of individual peaks at 520 nm, while pH differential measured the difference in sample absorbance of pH 1.0 and pH 4.5 at 510 nm. It is also possible that the polymeric pigments present in the samples were retained in the HPLC column and not included in HPLC measurements, whereas they might have contributed to the pH differential results.

Table 2 shows the color measurements performed on the juice and concentrate samples. The L* values ranged from 23.3 to 36.4 for the pasteurized juices, and from 23.6 to 37.4 for concentrates. The heat-treated samples were darker (smaller L* values) than the control and SO_2-treated samples. Hue angles (h) ranged from 10.9 to 17.7 and chroma (C) ranged from 43.1 to 49.9. All pasteurized juices had a higher hue angle and chroma than their concentrates. Both treated pasteurized juice samples had a more intense color (higher chroma) than control pasteurized juice. The concentration step shifted the pasteurized juice samples from red towards red-purple (shift in hue angle) for the 3 different processes.

SO_2 levels and pH

There was 8.4 ppm of total SO_2 present in the single-strength SO_2-treated pasteurized juice, and 8.1 ppm present in the single-strength concentrate, which is below the maximum level of SO_2 (10 ppm) for labeling (Title 21, U.S. Code of Federal Regulations 101.100).

From all 3 treatments, pasteurized juice samples had a pH of 3.92 to 4.07. The diluted concentrate samples (diluted to 13.5 °Brix) had a pH of 3.84 to 4.09.

Characterization of blueberry polyphenolics

The HPLC chromatogram of the ethyl acetate fraction revealed chlorogenic acid (strong 320 nm absorber) and 3 flavonol-glycosides (quercetin-3-glucoside and 2 other flavonol-glycosides, strong 260 nm absorber). Chlorogenic acid was the major cinnamic acid present (65% of polyphenolics) in Rubel blueberries.

Figure 3 shows the HPLC separation of the saponified ethyl acetate fraction monitored at 260, 280, and 320 nm. Alkaline hydrolysis of the ethyl acetate fraction revealed p-hydroxybenzoic acid, vanillic acid, chlorogenic acid, caffeic acid (released from chlorogenic acid), syringic acid, ferulic acid, o-coumaric acid, quercetin-3-glucoside, and 2 other flavonol-glycosides. Other researchers (Skrede and others 2000; Prior and others 2001) have reported the presence of catechin and epicatechin in highbush blueberries. Catechin and epicatechin were not detected in these Rubel extracts. Peak assignments were made according to the UV-visible spectra, retention time, and co-chromatography with authentic standards. Several workers have reported chlorogenic acid as the major cinnamic acid present, and also the greatest contributor of total polyphenolic content in highbush blueberries (Schuster and Hermann 1985; Gao and Mazza 1994; Kader and others 1996; Kalt and McDonald 1996; Häkkinen and others 1999; Kalt and others 2000; Skrede and others 2000). Quercetin- and kaempferol-glycosides were also detected. Quercetin-3-glucoside was the major flavonol-glycoside present. Azar and others (1987), Kader and others (1996), and Häkkinen and others (1999) reported quercetin-3-rhamnoside, quercetin-3-galactoside, quercetin-3-glucoside, kaempferol-3-glucoside, myricetin, caffeic acid and quinic acid (released from chlorogenic acid), chlorogenic acid, p-coumaric acid, ferulic acid, syringic acid, gallic acid, protocatechuic acid, p-hydroxybenzoic acid, m-hydroxybenzoic acid, vanillic acid, m-coumaric acid, and o-coumaric acid present in bilberries using HPLC, TLC, acid hydrolysis, and alkaline hydrolysis. Prior and others (2001) noted the presence of B_1 through B_8 procyanidin oligomers and monomers corresponding to (+)-catechin and (-)-epicatechin.

The concentration of polyphenolics was found to be 175.8 mg/100 g of frozen berries. Prior and others (1998) found 390.5 mg of polyphenolics/100 g of fresh berries

Table 2—Total monomeric anthocyanin content, % polymeric color, color measurements and actual °Brix of pasteurized juice and concentrate samples.

		Control		Heat treatment		SO_2 treatment	
	Frozen fruit	Pasteurized juice	Concentrate	Pasteurized juice	Concentrate	Pasteurized juice	Concentrate
Total monomeric anthocyanin[1] (mg /100 g)	192.4	31.9	31.8	50.3	46.6	46.7	46.5
% Polymeric color	37.6	50.4	54.3	40.7	40.9	42.1	38.8
Color measurements[2]							
L*		36.4	36.3	23.3	23.6	34.1	37.4
C		44.0	43.1	49.3	48.8	49.9	47.3
h		15.4	13.2	17.7	15.5	12.8	10.9
Actual °Brix		14.2	70.8	15.0	71.3	13.5	66.8

[1]Total monomeric anthocyanin content was expressed as cyd-3-glu, e = 26,900 L cm^{-1} mol^{-1}, and MW = 449.2 g mol^{-1}. Units are mg /100 g of juice processing staring material. To obtain single-strength 10 °Brix standard for juice samples, multiply by 0.618. To obtain 65 °Brix for concentrate, multiply value by 4.02.
[2]All samples were diluted to 10 °Brix. Hunter colorimeter was used (Illuminant C, 10 ° observer angle, and 0.25 cm cell were used).

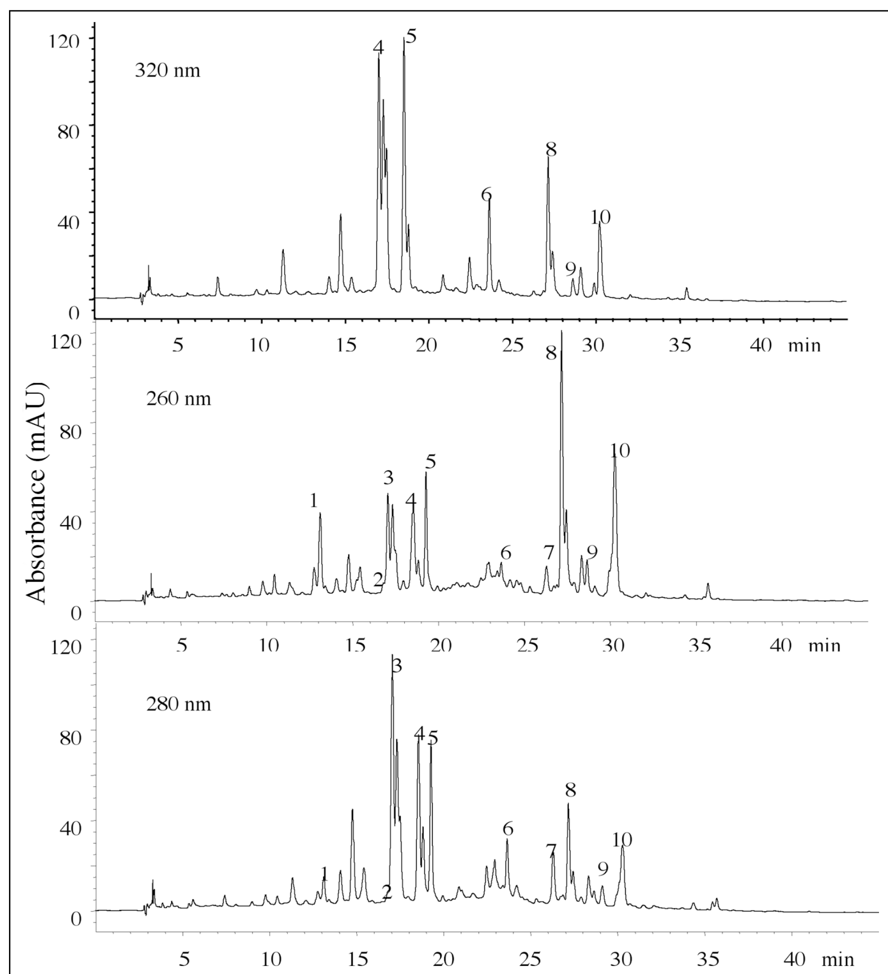

Figure 3—HPLC chromatogram of the saponified ethyl acetate fraction (polyphenolics). 1: p-hydroxybenzoic acid, 2: vanillic acid, 3: chlorogenic acid, 4: caffeic acid, 5: syringic acid, 6: ferulic acid, 7: o-coumaric acid, 8: quercetin-3-glucoside, 9: flavonol-glycoside, and 10: flavonol-glycoside

Table 3—Total polyphenolics content in the different samples during the 3 juice processing trials (C: control, H: heat treatment, and S: SO_2 treatment)[1]

	Presscake	Pressed juice				Clarified juice				Pasteurized juice			Concentrate		
	Fruit	C	H	S	C	H	S	C	H	S	C	H	S	C	H	S
Cinnamic acids	114.9	37.5	40.8	38.1	40.1	38.5	38.6	39.5	39.4	40.4	39.3	39.2	40.7	14.4	17.4	11.1
Flavonol-glycosides	60.9	23.2	29.0	26.0	26.9	30.0	26.2	23.7	28.2	28.4	22.1	28.0	25.7	21.5	16.6	16.1
Total[2]	175.8	60.7[a]	69.7[a]	64.1[a]	66.9[a]	68.5	64.8[a]	63.2[a]	67.6[a]	68.8[a]	61.4[a]	67.2[a]	66.5[a]	35.9[a]	34.0[a]	27.3[a]
SD	1.6	10.1	2.3	2.9	7.0	3.7	6.8	6.1	3.9	10.7	6.5	2.7	12.6	5.8	1.2	3.0

[1]Units are mg of polyphenolics/100 g of berries (starting material).
[2]Total with different lower case letters were significantly different ($p < 0.05$). To obtain single-strength 10 °Brix for juice samples, multiply by 0.618. To obtain 65 ºBrix for concentrate, multiply value by 4.02.

(expressed as gallic acid equivalents) in Rubel berries grown in Michigan, by the Folin-Ciocalteu method. Ehlenfeldt and Prior (2001) reported Rubel to have 1.65 mg of total phenolics/g of fresh weight (expressed as gallic acid equivalents). Gao and Mazza (1994) reported that 50 to 100 mg/100 g of chlorogenic acid was present in lowbush and highbush blueberries, and Wang and Jiao (2000) found chlorogenic acid to be an active antioxidant. Prior and others (2001) reported the total procyanidin content of Rubel was 6 μg/g (dry weight basis), while Skrede and others (2000) found 10 mg of procyanidins/100 g of frozen Bluecrop blueberries.

Changes in polyphenolics during juice processing

For simplicity, the individual compounds were summed as cinnamic acids and flavonol-glycosides. Table 3 shows the changes in the content of polyphenolics from each sample during the 3 juice processing trials. The starting material (frozen berries) had 175.8 mg/100 g of polyphenolics. There was considerable loss from the initial steps of processing: 60 to 65% of the blueberries' polyphenolics was lost during thawing, crushing, and pressing. Similar results were also reported by Skrede and others (2000). The polyphenolic content of samples taken at each step of heat and SO_2 treatments were not significantly different from the control ($p < 0.05$), which was an unexpected result. A possible explanation is that native enzymes (such as peroxidase), in addition to PPO, may also have degraded anthocyanins and, to a lesser extent, polyphenolics. Control pressed juice and SO_2-treated pressed juice had a lower value of polyphenolics than their pasteurized juices, but this was not the case for heat treatment. The presscakes held 15 to 20% of the frozen berries' polyphenolics, which is less than the proportion of anthocyanins left in the presscakes. Thirty-six to 39% of the polyphenolics in the berries was present in the pasteurized juice. Less than 3% of the polyphenolics in the pasteurized juices were lost during concentration. Forty-two to 45% (74.2 to 79.7 mg/100 g) of the frozen berries polyphenolics was lost during juice processing and not accounted in the final pasteurized juices and presscakes.

Cinnamic acids

The identified cinnamic acid's peaks were 65.4% of the total peak area in fresh fruit measured at 320nm. Thirty-three to 36% of the frozen berries cinnamic acids was extracted into the pressed juice. There were only slight changes in the subsequent juice processing steps. The pasteurized juice contained only 34 to 35% of the frozen berries' cinnamic acid. The presscakes held 10 to 15% of the original cinnamic acid content.

Flavonol-glycosides

The identified flavonol-glycoside's peaks represented 25.7% of the total peak area in fresh fruit at 260 nm. Thirty-eight to 48% of the frozen berries flavonol-glycosides was extracted into the pressed juice. There were minor changes in the flavonol-glycoside content of samples following the pressing step. Thirty-nine to 47% of the Rubel flavonol-glycosides was in the pasteurized juice. Presscakes contained 26 to 35% of the starting material's flavonol-glycoside content.

Häkkinen and others (2000) reported that black currant juices made with common domestic processing procedure resulted in a considerable loss of flavonols, and that cold-pressing was more efficient than steam-extraction in recovering flavonols. Processing aggravated the progressive enzymatic or chemical oxidation of phenolic compounds.

Conclusion

There was considerable loss of anthocyanins and polyphenolics in treatments and control during thawing, crushing, depectinization, and pressing steps of juice and concentrate processing. Heat and SO_2 treatments yielded higher anthocyanin content in each processing step, and should offer attractive attributes to traditional blueberry juice processing methods for increasing anthocyanin recovery. There were no differences in polyphenolic contents among the treatments and control. These results suggest that antioxidant activity of blueberry juice and concentrate were considerably lower than the whole blueberry fruit. There were low losses of anthocyanins and polyphenolics during the clarification and concentration steps. The presscake contained a substantial amount of anthocyanins and polyphenolics, and would be a rich source for natural antioxidants and natural colorants. These findings will be useful to processors wishing to improve the final yield of flavonoids and the antioxidant capacity in their blueberry products.

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