Anthocyanin and Polyphenolic Composition of Fresh and Processed Cherries

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ABSTRACT: The distribution of anthocyanin pigments and polyphenolics in 1 sour cherry (Prunus cerasus) and 3 sweet cherry (Prunus avium) cultivars was determined by high-performance liquid chromatography with diode array detection. Changes during frozen storage, canning, and brining were also monitored. Sweet cherry cultivars differed qualitatively with respect to the minor anthocyanins. Hydroxycinnamates are the major class of polyphenolics in sweet cherries, whereas flavanols are in the majority in Montmorency cherries. Hydroxycinnamates were greatly affected by processing and storage, whereas flavonol glycosides were quite stable. Half of the anthocyanins and polyphenolics were transferred to the syrup with canning, and nearly all were transferred to brine during brining.

Keywords: anthocyanins, polyphenolics, canning, brining, frozen storage

Introduction

It is widely accepted that a diet rich in fruits and vegetables will reduce the risk of several oxidative stress diseases, including coronary heart disease, cancer, stroke, and dementia. These health benefits are ascribed to phytochemicals such as carotenoids and polyphenolics, which include anthocyanin pigments, flavan-3-ols, procyanidins, flavonol glycosides, phenolic acids, and ellagic-acid derivatives (Ames and others 1993; Halliwell and others 1995; Hortog and others 1995; Porter and others 2001; Aviram and Fuhrman 2002; Bors and Michel 2002). Our laboratory has recently investigated the anthocyanin pigment and total phenolic contents of cherries along with their antioxidant properties (Chaovanalikit and Wrolstad 2003). The antioxidant properties of sour cherries have been extensively investigated by Michigan workers (Wang and others 1999c; Wang and others 2000; Seeram and others 2002).

The major anthocyanin pigments in sweet and sour cherries have been identified. Cyanidin-3-rutinoside, cyanidin-3-glucoside, peonidin-3-rutinoside, peonidin-3-glucoside, and pelargonidin-3-rutinoside have been identified in Bing and other sweet cherry cultivars (Lynn and Luh 1964; Gao and Mazza 1995; Mozetic and others 2002). Cyanidin-3-glucosyrutinoside, cyanidin-3-rutinoside, cyanidin-3-glucoside, and cyanidin-3-sophoroside have been identified in sour cherries (Dekazos 1970; Chandra and others 1992); cyanidin-3-arabinosylrutinoside, cyanidin-3-gentiobioside, and peonidin-3-rutinoside have also been reported to be present (Schaller and Von Elbe 1968; Dekazos 1970; Shrikhande and Francis 1973a; Chandra and others 2001).

Hydroxycinnamates are a major class of polyphenolics that are present in cherries. Schaller and Von Elbe (1970) identified 6 isomers of caffeoylquinic acid, 4 isomers of p-coumaroylquinic acid, caffeic acid, and p-coumaric acid in Montmorency cherries. Neochlorogenic acid and 3'-p-coumaroylquinic acid have been identified in sweet cherries (Gao and Mazza 1995; Friedrich and Lee 1998; Mozetic and others 2002). The presence of caffeoyltartaric acid in sweet and sour cherries and 4'-p-coumaroylquinic acid in sour cherries has also been reported (Friedrich and Lee 1998). Flavonols and flavonol glycosides are another important class of cherry polyphenolics, the rutinosides and glucosides of quercetin and kaempferol being reported by several workers in sweet and sour cherries (Schaller and Von Elbe 1970; Shrikhande and Francis 1973b; Henning and Herrmann 1980). In addition, Shrikhande and Francis (1973b) reported the presence of quercetin-4'-glucoside, kaempferol-3-rhamnoside-4'-galactoside (tentative), and kaempferol-4'-glucoside (tentative). Henning and Herrmann (1980) isolated the 3,4'-diglucosides of kaempferol and quercetin from sweet cherries and identified the galactoside of quercetin and kaempferol and the rhamnoside of quercetin in cherries. Friedrich and Lee (1998) found the flavonol epicatechin in both sweet and sour cherries and procyanidins in sour cherries.

Although all cherry cultivars have some anthocyanin pigmentation in the skins, the amounts vary tremendously (Chaovanalikit and Wrolstad 2003). Some varieties have pigmentation in the flesh, for example, Bing, and others do not, for example, Royal Ann and Montmorency. The distribution of individual phenolics and polyphenolic classes in skins, flesh, and pits has not been reported. Pits in particular are a substantial cherry-processing waste and could be a potential source of polyphenolics for nutraceutical or antioxidant use.

We recently reported how total anthocyanins, total phenolics, and antioxidant properties of sweet cherries are altered when sweet cherries are processed by canning and brining and stored at freezing temperatures (Chaovanalikit and Wrolstad 2004). Processing clearly had an impact on all 3 parameters. In some instances, antioxidant properties actually increased, while total phenolics showed little change. Knowing how individual compounds and polyphenolic classes are affected by processing may help to understand the relationship between polyphenolics and their antioxidant properties.

Our objectives were to identify the anthocyanins and polyphenolics in sweet and sour cherries and to measure their distribution in skins, flesh, and pits. In addition, we would like to monitor how they were qualitatively and quantitatively affected with frozen storage, canning, and brining.

Materials and Methods

Standards

Phenolic standards (protocatechuic acid,
Cherry anthocyanin and polyphenolic composition...

Chlorogenic acid, epicatechin, caffeic acid, p-coumaric acid, and quercetin-3-rutinoside) were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Quercetin-3-glucoside, quercetin-3-rhamnoside, kaempferol-3-glucoside, and kaempferol-3-rutinoside were purchased from Extrasynthese (Genay, France). Concord grape juice concentrate (Welch Food Inc., Concord, Mass., U.S.A.) was purchased at a local supermarket, whereas cranberry, black currant, red raspberry, and strawberry juice concentrates were provided by Kerr Concentrates Inc. (Salem, Ore., U.S.A.).

Sources of cherry samples

Rainier sweet cherries were provided by the Mid-Columbia Experiment Station, Hood River, Ore. Two varieties of sweet cherries, Bing and Royal Ann, and 1 variety of sour cherries, Montmorency, were harvested at the Lewis Brown Horticultural Farm, Oregon State Univ. Dept. of Horticulture, Corvallis, Ore. Oregon Cherry Growers, Inc., Salem, Ore., supplied Bing and Royal Ann cherries for the brining experiment and also provided Bing cherries for canning and frozen storage experiments. Cherries were delivered to the Dept. of Food Science and Technology, Oregon State Univ., Corvallis, Ore., and then were stored at 2 °C before sample preparation or processing.

Fresh cherries

Cherry samples (250 g) were separated by hand into skins, flesh, and pits. The skins, flesh, and pits were frozen separately in liquid nitrogen, weighed, and kept at −70 °C until further analysis. The samples were prepared in 2 replicates.

Cherry processing

Frozen cherries. Stemmed Bing cherries (2 kg) were washed, destemmed, and pitted by a household hand cherry pitter. Pitted cherries were then frozen in liquid nitrogen. Samples (120 g) were packaged into 12 plastic Nalgene containers (Nalge Nunc Intl., Rochester, N.Y., U.S.A.), closed, and stored at 22 °C. After 12 mo, cherries were washed with running cold water for about 5 d, until sulfur dioxide content of cherries was less than 200 parts per million. Washed pitted cherries were frozen with liquid nitrogen and stored at −70 °C. The spent brine solution was also stored at −70 °C.

Extraction of anthocyanins and polyphenolics

Samples were liquid nitrogen powdered using a stainless-steel Waring Blender. Powdered samples (about 10 g) were blended with 20 mL of acetone, sonicated (Ultrasonic cleaner, Branson Cleaning Equipment Corp., Shelton, Conn., U.S.A.) for 10 min, and then filtered on a Büchner funnel using Whatman nr 1 paper (Whatman Inc., Clifton, N.J.). The filter cake was re-extracted with 10 mL of 70% acetone (30% water and 70% acetone, v/v) twice. Filtrates were combined, mixed with 80 mL of chloroform, and then centrifuged at 170 × g for 20 min by IEC Int'l. Centrifuge (Model UV, Intl. equipment Co., Boston, Mass., U.S.A.). The aqueous phase was collected and evaporated in vacuo at 40 °C until the residual acetone residue was removed (about 10 min). The fraction was made up to 25 mL with acidified water and stored at −70 °C until further analysis. Sample extractions were replicated twice.

Anthocyanin and polyphenolic purification

The purification of anthocyanins and polyphenolics was conducted as described by Durst and Wrolstad (2001). The anthocyanin or polyphenolic isolate (about 1 mL) was saponified in a screw-cap test tube with 5 mL of 10% KOH for 8 min in the dark at room temperature. Then, 5 mL of 2 N HCl was added to neutralize the solution. The hydrolysate was purified by solid-phase extraction using a C-18 Sep-Pak cartridge (Waters Assoc.) as previously described.

Acid hydrolysis of anthocyanins

Acid hydrolysis was performed by the procedure as described by Durst and Wrolstad (2001). Five milliliters of 2 N HCl were added to purified anthocyanins (about 1 mL) in a screw-cap test tube, flushed with nitrogen, and capped. The purified compound was hydrolyzed for 30 min at 100 °C and then immediately cooled in an ice bath. The hydrolysate was purified by solid-phase extraction using a C-18 Sep-Pak cartridge (Waters Assoc.) as described earlier.

Alkaline hydrolysis of anthocyanins and polyphenolics

The saponification was performed by the procedure as described by Durst and Wrolstad (2001). The anthocyanin or polyphenolic isolate (about 1 mL) was saponified in a screw-cap test tube with 5 mL of 10% KOH for 8 min in the dark at room temperature. Then, 5 mL of 2 N HCl was added to neutralize the solution. The hydrolysate was purified by solid-phase extraction using a C-18 Sep-Pak cartridge (Waters Assoc.) as described earlier.

HPLC analytical system

A high-performance liquid chromatography Perkin Elmer Series 400 (Norwalk, Conn., U.S.A.), equipped with a Hewlett-Packard 1040A photodiode array detector and Gateway 2000 P5-90 computer with Hewlett-Packard HPLC® Chemstation software (Waldbron, Germany) was used with simultaneous detection at 520 nm for anthocyanins, saponified anthocyanins, and anthocyanidins and at 260, 280, 320, 370, and 520 nm for saponified phenolics and phenolic characterization and quantifica-
HPLC separation of anthocyanins and saponified anthocyanins

Anthocyanins and saponified anthocyanins were separated using a Prodigy ODS-3 column (5 μm) 250 mm × 4.6-mm inner dia (Phenomenex, Torrance, Calif., U.S.A.) fitted with an Allsphere 10 mm × 4.6-mm inner dia ODS-2 guard column (Altech, Deerfield, Ill., U.S.A.). Solvent A was 100% HPLC-grade acetonitrile and solvent B was 1% phosphoric acid, 10% acetic acid (glacial), and 5% acetonitrile (v:v:v) in water. The program was isocratic at 0% A for 5 min, a linear gradient from 0% to 20% A for 15 min, and a linear gradient from 20% to 40% A for 5 min. Identification was made from matching UV-visible spectra and retention times with known anthocyanins from fruit juice standards.

HPLC separation of anthocyanidins

Anthocyanidin separation used a Prodigy ODS-3 column (5 μm) 250 mm × 4.6-mm inner dia (Phenomenex), fitted with an Allsphere 10 mm × 4.6-mm inner dia ODS-2 guard column (Altech). Solvents A and B, as described previously for anthocyanins, were used. The program was a linear gradient from 10% to 30% A for 20 min. Identification was made from matching UV-visible spectra and retention times with 6 anthocyanidins obtained from acid hydrolysis of grape and strawberry juice concentrates.

HPLC separation of polyphenolics and saponified polyphenolics

Polyphenolics were separated using a Synergi Hydro-RP (4 μm) 250 mm × 4.6-mm inner dia (Phenomenex) fitted with an Allsphere 10 mm × 4.6-mm inner dia ODS-2 guard column (Altech). Solvent A was 100% HPLC-grade acetonitrile, whereas solvent B was 1% acetic acid in deionized water. The program was isocratic at 5% A for 5 min, a linear gradient from 5% to 25% A for 30 min, then a linear gradient from 25% to 50% A for 3 min, and finally isocratic at 50% A for 5 min. Identification was made by matching the UV-visible spectra and retention time with authentic standards (when available).

HPLC quantification of polyphenolics

Phenolics were separated using a Prodigy ODS-3 column (5 μm) 250 mm × 4.6-mm inner dia (Phenomenex), fitted with an Allsphere 10 mm × 4.6-mm inner dia ODS-2 guard column (Altech). Solvent A was 100% HPLC-grade acetonitrile while solvent B was 0.07 M KH₂PO₄ adjusted to pH 2.4 with concentrated H₃PO₄. The program was isocratic at 5% A for 3 min, a linear gradient from 5% to 35% A for 27 min, and then a linear gradient from 35% to 55% A for 5 min. Hydroxycinnamates were quantified by the external standard method as chlorogenic acid at 320 nm, epicatechin and procyanidins as epicatechin at 280 nm, and flavonol glycosides as queretin-3-rutinoside (rutin) at 260 nm.

Electrospray mass spectrometry

Low-resolution mass spectrometry was obtained using electrospray mass spectrometry (ESMS). The instrument was a Perkin Elmer SCIEX API III bimolecular mass analyzer (Thornhill, Ont., Can.) equipped with an ion spray interface (ISV = 4000, orifice voltage = 60). The mass spectrometer was operated in the positive mode. Purified anthocyanin and purified polyphenolic fractions were introduced into the ESMS by a 100-μL glass syringe connected with the infusion pump at the rate of 12.55 μL/min.

Statistical analyses

Effects of processing on cherry anthocyanin peak area and polyphenolic composition were separately analyzed by analysis of variance using S-Plus 4.5 (MathSoft, Seattle, Wash., U.S.A.). To assess the effect of frozen storage on anthocyanin peak area and individual phenolic content, a 1-factor ANOVA model was used, the factor being fresh cherries and the combination of frozen temperature and storage time. To evaluate the effect of canning on individual phenolic content, a 1-factor ANOVA model was used with the levels of the factors as follows: fresh cherries and canned cherries at 2 levels of storage time. To evaluate the effect of freezing on individual phenolic content, a 2-factor ANOVA model was used with the factors as follows: time and temperature. The pairwise comparison between significant treatment level means in all ANOVA models was carried out using Tukey’s method (P ≤ 0.05). To compare the mean of anthocyanin peak area in fresh cherries and canned cherries or spent brined solution for each cherry variety, a test was used to analyze separately. The Pearson correlation was used to determine the correlation among antioxidant activities and polyphenol composition in skins, flesh, and pits of cherries.
Results and Discussion

Anthocyanin composition of cherries

Eight different anthocyanins were identified in sweet and sour cherries (Table 1). Peak assignments are based on matching UV-visible spectra and retention times with anthocyanins from fruit juices that have been well characterized: cyanidin-3-glucoside, cyanidin-3-rutinoside, and peonidin-3-rutinoside from blackcurrants (Slimestad and Solheim 2002); cyanidin-3-sophoroside and cyanidin-3-glucosylrutinoside from red raspberries (Spanos and Wrolstad 1987); pelargonidin-3-glucoside and pelargonidin-3-rutinoside from strawberries (Bridle and García-Viguera 1997); and peonidin-3-glucoside from cranberries (Hong and Wrolstad 1990b). HPLC analysis of the acid hydrolysis products of the anthocyanin isolates showed cyanidin to be the major anthocyanin with trace amounts of peonidin and pelargonidin. Saponification showed no change in the anthocyanin pigment profile, indicating that none of the anthocyanins was acylated with cinnamic or aliphatic organic acids. In addition, electrospray mass spectra confirmed the identities of cyanidin-3-glucoside (m/z 449), cyanidin-3-rutinoside (m/z 595), cyanidin-3-sophoroside (m/z 611), cyanidin-3-glucosylrutinoside (m/z 757), pelargonidin-3-rutinoside (m/z 579), and peonidin-3-rutinoside (m/z 609).

The % relative intensity of minor pigments was insufficient to get definitive mass spectra. These assignments confirmed identifications by previous workers (Li and Wagenknecht 1958; Lynn and Luh 1964; Dekazos 1970; Shrikhande and Francis 1973a; Chandra and others 1992; Gao and Mazza 1995 and Mozetic and others 2002) with the exception that this is the 1st report for the presence of pelargonidin-3-glucoside.

Cyanidin-3-rutinoside is the major anthocyanin in sweet cherries, with cyanidin-3-glucoside being second. Sour cherries have a very different profile with cyanidin-3-glucosylrutinoside being the major pigment and cyanidin-3-rutinoside the 2nd largest pigment. This is consistent with previous reports (Hong and Wrolstad 1990a; Gao and Mazza 1995; Chandra and others 2001; Mozetic and others 2002). The 3 sweet cherry cultivars vary with respect to trace anthocyanins. Cyanidin-3-sophoroside was found in Royal Ann and Rainier cherries but not in Bing cherries. Pelargonidin-3-glucoside and peonidin-3-glucoside were found in Bing cherries but not in Royal Ann and Rainier cherries. Peonidin-3-rutinoside was not detected in Royal Ann cherries. To our knowledge, this is the 1st report on the anthocyanin composition of Rainier and Royal Ann cherries.

Gao and Mazza (1995) and Mozetic and others (2002) identified the anthocyanins of sweet cherries as cyanidin-3-rutinoside, cyanidin-3-glucoside, pelargonidin-3-rutinoside, peonidin-3-rutinoside, and peonidin-3-glucoside. Peonidin-3-glucoside was not detected in the light-colored sweet cherries (Gao and Mazza 1995). Sour cherries contained cyanidin-3-sophoroside and cyanidin-3-glucosylrutinoside along with most anthocyanins present in sweet cherries except for pelargonidin-3-rutinoside (Dekazos 1970; Hong and Wrolstad 1990a; Chandra and others 1992). Cyanidin-3-arabinoxylyrutinoside has been found in Balaton cherries (Chandra and others 2001).

Bing is the only cultivar containing anthocyanins in skins, flesh, and pits, with the skins being richest in anthocyanin concentration (Table 1). Figure 1 shows the HPLC anthocyanin profiles for Bing skins, flesh, and pits. The distribution pattern differs considerably, with the major anthocyanin of skins and flesh being cyanidin-3-rutinoside (about 79% and 56%), whereas the major anthocyanin in Bing pits was cyanidin-3-glucoside (about 51%). Pits contained much higher proportions of peonidin-3-glucoside and peonidin-3-rutinoside. Anthocyanins are not present in the flesh of Montmorency, Royal Ann, and Rainier cherries. Skins have the highest anthocyanin concentration, but pigments are also found in the pits. The distribution between skins and pits is different with the pits containing less cyanidin-3-rutinoside and more cyanidin-3-glucoside. Moreover, the distribution is different with cyanidin-3-sophoroside being present in much higher proportions in Montmorency pits than skins, whereas cyanidin-3-sophoroside is not present in Rainier and Royal Ann pits.

Polyphenolic composition of cherries

The polyphenolics were separated from the anthocyanins by solid-phase extraction on C-18 resin using ethyl acetate. Polyphenolics are eluted with ethyl acetate, whereas the anthocyanins remain absorbed until being removed by acidified methanol. A large number of peaks were separated by HPLC as illustrated by Figure 2 and 3, showing HPLC
phenolics were reported as mg gallic acid/100 g fresh weight (fw). Oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) values, which were reported in a previous publication (Chaovanalit and Wrolstad 2003).

Table 2—Total phenolic content, % area quantified, hydroxycinnamates, epicatechin, procyanidins, flavonol glycosides, ORAC, and FRAP in skins, flesh, and pits of sweet cherries and sour cherriesa

<table>
<thead>
<tr>
<th>Cultivarb</th>
<th>Portion</th>
<th>TPC</th>
<th>% Area</th>
<th>Hydroxycinnamates (mg chlorogenic acid/100 g)</th>
<th>Epicatechin (mg epicatechin/100 g)</th>
<th>Procyanidins (mg epicatechin/100 g)</th>
<th>Flavonol glycosides (mg rutin/100 g)</th>
<th>ORACb</th>
<th>FRAPb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bing</td>
<td>Edible</td>
<td>185.0</td>
<td>—</td>
<td>56.8</td>
<td>8.29</td>
<td>nd</td>
<td>4.06</td>
<td>1494</td>
<td>1590</td>
</tr>
<tr>
<td></td>
<td>Skins</td>
<td>333</td>
<td>99.6 ± 0.03</td>
<td>144 ± 7.21</td>
<td>18.5 ± 1.07</td>
<td>nd</td>
<td>17.2 ± 1.51</td>
<td>2626</td>
<td>2105</td>
</tr>
<tr>
<td></td>
<td>Flesh</td>
<td>134</td>
<td>93.1 ± 0.9</td>
<td>48.2 ± 6.03</td>
<td>7.71 ± 0.70</td>
<td>nd</td>
<td>1.59 ± 0.21</td>
<td>907</td>
<td>728</td>
</tr>
<tr>
<td></td>
<td>Pits</td>
<td>92</td>
<td>65.2 ± 3.5</td>
<td>8.53 ± 2.02</td>
<td>2.51 ± 0.49</td>
<td>4.70 ± 0.81</td>
<td>nd</td>
<td>594</td>
<td>504</td>
</tr>
<tr>
<td>Royal Ann</td>
<td>Edible</td>
<td>229</td>
<td>—</td>
<td>87.0</td>
<td>11.9</td>
<td>nd</td>
<td>20.2</td>
<td>1449</td>
<td>1553</td>
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<tr>
<td></td>
<td>Skins</td>
<td>351</td>
<td>98.4 ± 0.3</td>
<td>196 ± 11.79</td>
<td>19.6 ± 2.49</td>
<td>24.6 ± 2.75</td>
<td>20.64 ± 3.19</td>
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<td>1708</td>
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<td>Flesh</td>
<td>176</td>
<td>97.0 ± 0.3</td>
<td>83.0 ± 1.02</td>
<td>13.5 ± 0.52</td>
<td>25.3 ± 1.13</td>
<td>nd</td>
<td>1310</td>
<td>903</td>
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<td></td>
<td>Pits</td>
<td>104</td>
<td>63.2 ± 8.5</td>
<td>14.5 ± 1.28</td>
<td>2.70 ± 0.25</td>
<td>7.34 ± 0.88</td>
<td>nd</td>
<td>568</td>
<td>498</td>
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<tr>
<td>Rainier</td>
<td>Edible</td>
<td>75</td>
<td>—</td>
<td>70 ± 3.0</td>
<td>3.35</td>
<td>nd</td>
<td>1.15</td>
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<td>Skins</td>
<td>142</td>
<td>93.8 ± 3.1</td>
<td>72.1 ± 2.63</td>
<td>4.70 ± 0.75</td>
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<td>592</td>
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<tr>
<td></td>
<td>Flesh</td>
<td>65</td>
<td>90.3 ± 0.8</td>
<td>28.4 ± 0.37</td>
<td>3.77 ± 0.44</td>
<td>9.90 ± 0.44</td>
<td>nd</td>
<td>462</td>
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<tr>
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<td>Pits</td>
<td>54</td>
<td>68.1 ± 0.8</td>
<td>5.58 ± 0.38</td>
<td>1.22 ± 0.17</td>
<td>4.83 ± 0.56</td>
<td>nd</td>
<td>338</td>
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<tr>
<td>Montmorency</td>
<td>Edible</td>
<td>407</td>
<td>—</td>
<td>58.2</td>
<td>19.6</td>
<td>nd</td>
<td>49.5</td>
<td>11.2</td>
<td>2557</td>
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<tr>
<td></td>
<td>Skins</td>
<td>558</td>
<td>98.4 ± 0.1</td>
<td>139 ± 2.24</td>
<td>50.5 ± 2.61</td>
<td>108 ± 4.00</td>
<td>63.2 ± 4.17</td>
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<td>4796</td>
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<tr>
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<td>Flesh</td>
<td>301</td>
<td>97.1 ± 1.3</td>
<td>55.7 ± 0.45</td>
<td>17.7 ± 1.25</td>
<td>50.3 ± 5.13</td>
<td>nd</td>
<td>1500</td>
<td>1381</td>
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<tr>
<td></td>
<td>Pits</td>
<td>157</td>
<td>70.6 ± 0.7</td>
<td>12.7 ± 0.23</td>
<td>8.15 ± 0.38</td>
<td>22.5 ± 1.86</td>
<td>nd</td>
<td>997</td>
<td>848</td>
</tr>
</tbody>
</table>

Each value in table represents mean ± standard deviation (n = 2); nd = not detected.

Amount in the edible portion (skins plus flesh) was calculated from the proportionate weight of skins and flesh except for total phenolics, oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) values, which were reported in a previous publication (Chaovanalikit and Wrolstad 2003). Total phenolics were reported as mg gallic acid/100 g fresh weight (fw).

% Area denotes the percentage of the total peak area that was measured, for example, unidentified peaks accounts for 6.9% of the total peak area for Bing flesh.

Oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) were reported as μmoles Trolox equivalent/100 g fw.

chromatograms of Bing and Montmorency polyphenolics, respectively. Peaks were characterized as to compound class according to their UV-visible spectra, and in some instances specific compounds were identified by matching retention time with authentic standards, for example, epicatechin, chlorogenic acid, quercetin-3-rutinoside, quercetin-3-glucoside, and kaempferol-3-rutinoside. The presence of epicatechin (m/z 291), chlorogenic acid (m/z 355), 3’-p-coumaroylquinic acid (m/z 339), kaempferol-3-rutinoside (m/z 595), and quercetin-3-rutinoside (m/z 611) in sweet and sour cherries as well as the presence of quercetin-3-glucoside (465) in Bing and Montmorency skins were confirmed by Electrospray mass spectra. These peak assignments are consistent with previous identifications reported in the literature (Schaller and Von Elbe 1970; Gao and Mazza 1995; Friedrich and Lee 1998; Wang and others 1999a; Wang and others 1999b; Mozetic and others 2002).

The major classes of phenolic compounds for both sweet and sour cherries were hydroxycinnamates (determined as chlorogenic acid), epicatechin and procyanidins (determined as epicatechin), and flavonol glycosides (determined as rutin). Table 2 compares amounts of the different polyphenolics for skins, flesh, and pits of the 4 different cultivars. The polyphenolic content for the edible portion (skin plus flesh) is also shown because of its nutritional relevance. The percentage of total peak area representing the measured peaks is also listed. For example, the peak areas measured for nonanthocyanin polyphenolics in Bing skins represent 99.6% of the total peak area, whereas the nonanthocyanin polyphenolics in Bing pits were 65.2% of the total peak area, the remainder not being identified or quantitated.

All sweet cherry skins contained 9 hydroxycinnamates.
Cherry anthocyanin and polyphenolic composition...  

Hydroxycinnamate peaks, 1 flavanol peak, and 2 flavonol glycoside peaks. Bing contained an additional flavonol glycoside peak, whereas Royal Ann and Rainier contained an additional hydroxycinnamate peak (data not shown). Peak 3 was identified as chlorogenic acid according to its matching UV-visible spectra and retention time with authentic standards. Peak 1 had a matching UV spectrum to chlorogenic acid while Peak 2's UV spectrum matched p-coumaric acid. These peaks completely disappeared with saponification, with caffeic acid and p-coumaric acid being formed. Peak 1 was assigned to be neochlorogenic acid (3'-caffeoylquinic acid) and peak 2 as 3'-p-coumaroylquinic acid. This is consistent with previous reports (Gao and Mazza 1995; Friedrich and Lee 1998; Mozetic and others 2002). Gao and Mazza (1995) and Mozetic and others (2002) reported that the major phenolic of sweet cherries was neochlorogenic acid, whereas Friedrich and Lee (1998) reported that the major phenolic in sweet and sour cherries was caffeyl-tartaric acid, which was not evident from ESMS mass spectra data in our investigation. The remaining 6 peaks with spectra characteristic of hydroxycinnamates were measured as hydroxycinnamates.

Skins contained the highest concentrations of hydroxycinnamates with substantially less in the flesh (Table 2). Note that the amounts in the edible portion were slightly greater than in flesh by itself because the skins are a relatively small proportion of the fruits. Whereas pits were low in hydroxycinnamates compared with skins and flesh, they still accounted for a substantial proportion of the polyphenolics in sweet cherry skins. Of the quantified non-anthocyanin polyphenolics, the major hydroxycinnamates in sweet cherry skins, flesh, and pits were neochlorogenic acid (54% to 57%, 42% to 56%, and 25% to 31%), 3'-p-coumaroylquinic acid (6% to 10%, 13% to 18%, and 7% to 19%), and chlorogenic acid (4% to 5%, 1% to 12%, and 1.1% to 1.4%).

Montmorency skins contained 7 hydroxycinnamate peaks, 6 flavanol peaks, and 4 flavonol glycoside peaks (Figure 3), the major peaks being identified as 3'-p-coumaroylquinic acid (peak 2), chlorogenic acid (peak 3), and neochlorogenic acid (peak 1). The identification is in agreement with literature identifications (Schaller and Von Elbe 1970; Friedrich and Lee 1998). The major hydroxycinnamates in sour cherry skins, flesh, and pits were different. Of the quantified non-anthocyanin polyphenolics, the major hydroxycinnamates in sour cherry skins were chlorogenic acid (14.1%), 3'-p-coumaroylquinic acid (11.6%), and neochlorogenic acid (6.9%); in flesh and pits they were 3'-p-coumaroylquinic acid (24.8% and 18.5%), chlorogenic acid (8.95% and 4.72%), and neochlorogenic acid (8.15% and 3.49%).

Epicatechin was identified from matching UV-visible spectra and retention time with an authentic standard. It was present in all cultivars. The presence of epicatechin was in agreement with others (Friedrich and Lee 1998; Arts and others 2000). Epicatechin is another major polyphenolic in cherries, being found in all cultivars and in skins, flesh, and pits. As with the hydroxycinnamates, skins had the highest concentrations and the edible portion contained slightly more than the flesh alone.

Procyanidins were identified by matching UV-visible spectra with epicatechin (Bartolomé and others 1996). There were large cultivar differences with respect to procyanidins. No procyanidins were detected in Bing skins and flesh, whereas Royal Ann contained substantial quantities in both skins and flesh. Rainier contained traces of procyanidins in skins and moderate amounts in the flesh. Montmorency was particularly high in procyanidins in the skins. Cherry pits contained high proportion of procyanidins (29% to 52.5%). Procyanidin B2 has been identified in sour cherries (Friedrich and Lee 1998).

Three flavonol glycoside peaks in sweet and sour cherries were identified as quercetin-3-rutinoside, quercetin-3-glucoside, and kaempferol-3-rutinoside, which agrees with Shrikhande and Francis (1973b), Schaller and Von Elbe (1970), and Wang and others (1999b). Quercetin-3-rutinoside and kaempferol-3-rutinoside were found in all sweet and sour cherry cultivars with quercetin-3-glucoside being found only in Bing and Montmorency skins. Henning and Hermann (1980) also reported that the glycosides and rutinosides of both quercetin and kaempferol were present in sweet cherries. Kaempferol-3-glucoside and quercetin-3-rhamnoside have been reported for Montmorency cherries (Schaller and Von Elbe 1970; Wang and others 1999b), but we did not detect these compounds in our study by comparing retention time with authentic standards. There were additional unidentified peaks with characteristic flavonol spec-
Table 3—Relative anthocyanin composition of fresh and frozen Bing cherries stored at 3 mo and 6 mo at 2 different tempera-
tures (–23 °C and –70 °C)a

<table>
<thead>
<tr>
<th>Temp (°C)</th>
<th>Time (mo)</th>
<th>ACN (mg /100 g)</th>
<th>Cyanidin-3-glucoside</th>
<th>Cyanidin-3-rutinoside</th>
<th>Pelargonidin-3-glucoside</th>
<th>Pelargonidin-3-rutinoside</th>
<th>Peonidin-3-glucoside</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>—</td>
<td>63.7</td>
<td>8.05 ± 0.08a</td>
<td>85.2 ± 0.17a</td>
<td>0.56 ± 0.03c</td>
<td>nd</td>
<td>0.91 ± 0.02b</td>
<td>5.26 ± 0.09cd</td>
</tr>
<tr>
<td>Freezing</td>
<td>–23</td>
<td>3</td>
<td>8.90 ± 0.76a</td>
<td>80.3 ± 0.77b</td>
<td>1.35 ± 0.03b</td>
<td>0.29 ± 0.06b</td>
<td>1.05 ± 0.15a</td>
<td>8.11 ± 0.17a</td>
</tr>
<tr>
<td>Freezing</td>
<td>–23</td>
<td>6</td>
<td>7.56 ± 0.07a</td>
<td>80.8 ± 0.44b</td>
<td>3.06 ± 0.17a</td>
<td>0.65 ± 0.02a</td>
<td>0.94 ± 0.08ab</td>
<td>7.05 ± 0.17b</td>
</tr>
<tr>
<td>Freezing</td>
<td>–70</td>
<td>3</td>
<td>9.40 ± 1.12a</td>
<td>84.6 ± 0.81a</td>
<td>0.43 ± 0.01c</td>
<td>nd</td>
<td>0.79 ± 0.001b</td>
<td>4.84 ± 0.33d</td>
</tr>
<tr>
<td>Freezing</td>
<td>–70</td>
<td>6</td>
<td>8.36 ± 1.74a</td>
<td>83.9 ± 1.82a</td>
<td>0.46 ± 0.01c</td>
<td>nd</td>
<td>0.78 ± 0.07b</td>
<td>5.46 ± 0.13c</td>
</tr>
</tbody>
</table>

*aAnthocyanin contents were reported as mg cyanidin-3-glucoside/100 g fw (Chaovanalikit and Wrolstad 2003). Each value in table i s mean ± standard deviation (n = 2). The values with different letters in a column indicate a significant difference at α = 0.05.

A striking finding is that with the exception of Bings, flavonol glycosides were found only in the skins and not detected in flesh or pits (Table 2). The flavonol glycoside concentration in Bing flesh was relatively low.

Whereas the qualitative composition of sweet and sour cherries are similar, the proportions of polyphenolics are different (Table 2). In the edible portion (skin plus flesh), major compounds of sweet cherries were hydroxycinnamates (70.7% to 82.1%), epicatechin (8.0% to 12%), and flavonol glycosides (2.8% to 5.9%). Procyanidins were present in Royal Ann (16.4%) and Rainier (17.3%) cherries but not in Bing cherries. Montmorency cherries had the highest content of epicatechin (19.6 mg/100 g), procyanidins (49.5 mg/100 g), and flavonol glycosides (11.2 mg/100 g). Royal Ann cherries contained the highest content of hydroxycinnamates (87 mg/100 g). Within sweet cherries, Bing cherries contained the highest content of flavonol glycosides (4.1 mg/100 g), whereas Royal Ann cherries contained the highest epicatechin (11.9 mg/100 g) and procyanidins (20.2 mg/100 g). The major compounds of Montmorency cherries were hydroxycinnamates (42%), epicatechin (14.2%), procyanidins (35.7%), and flavonol glycosides (8.1%).

Values for total phenolics as determined by the Folin-Ciocalteu procedure are not in good agreement with values obtained by summing individual polyphenolic classes (Table 2) and total anthocyanins (Table 1); for example, total phenolics of Bing skin are 333 mg/100 g fresh weight, whereas the sum of total polyphenolic classes and anthocyanins are 241 mg/100 g. This is not surprising because the different phenolic classes contain different numbers of phenolic groups and would be expected to respond somewhat differently to the Folin-Ciocalteu reagent. Similar trends are shown, however, for total phenolics derived by the 2 different procedures (r = 0.97).

In this investigation, Bing cherries were used for processing experiments. The polyphenolic compositions of the different lots of Bing cherries are shown in Table 2, 4, and 6. The 2 different lots have similar proportions of different polyphenolics, but there is substantial difference in individual levels, for example, 57 (Table 2) compared with 74 (Table 4) and 70 (Table 6) mg/100 g of hydroxycinnamates. The levels of hydroxycinnamates, which we measured in Bing cherries, are much lower than the values reported in Gao and Mazza (1995), but higher than the amounts reported by Mozetic and others (2002). The proportions of individual hydroxycinnamates (the ratio of neochlorogenic acid to 3-p-coumaroylquinic acid) are similar.

Changes in anthocyanins and polyphenolics during frozen storage

Table 3 and 4 compare the anthocyanin and polyphenolic composition of fresh and
Formation of minor unidentified anthocyanin peaks was detected in cherries stored at −23 °C after 3 mo, and the amounts increased at 6 months. Possible anthocyanin degradation products are anthocyanin-polyphenolic condensation compounds (Escribano-Bailón and others 1996; Es-Safi and others 2002). Several investigators have shown that chlorogenic acid and epicatechin are favored substrates for polyphenoloxidase (Amiot and others 1995; Kader and others 1997). Other investigators have demonstrated that flavonol glycosides are affected by only a small degree by polyphenoloxidase. This degradation was minimal at −70 °C storage.

Changes in anthocyanins and polyphenolics during canning and canned storage

Table 5 shows the effect of canning and storage at 2 °C and 22 °C on the anthocyanin pigments of Bing cherries. Although the visual appearance of Bing cherries was markedly affected by canning, there was surprisingly no anthocyanin degradation. There was an apparent increase in total anthocyanins, which might be explained by increased extraction efficiency of the softened fruits. Ap-
approximately half of the anthocyanins were leached into the syrup with canning. The anthocyanin pigment profiles of fresh cherries, canned cherries, and canned syrup were similar; however, there were some apparent differences. The proportions of cyanidin and pelargonidin rutinosides in cherries slightly decreased with canning, whereas cyanidin and pelargonidin glucosides increased. Partial hydrolysis of rutinose to glucoside can account for these changes (Francis 1972; Withy and others 1993). Substantial anthocyanin degradation occurred after 5-mo storage at 2 °C (12%) and 22 °C (42%). Weinert and others (1990) showed similar anthocyanin losses for canned plums stored for 47 d at 4 °C (13.5%) and 30 °C (46%). The proportions of the individual anthocyanins were little affected by storage; however, there was some evidence especially in storage at 22 °C for partial hydrolysis of rutinosides to glucosides and also evidence that cyanidin-glycosides were more labile than pelargonidin-glycosides.

Table 6 shows how Bing polyphenolics are affected by canning and storage at 2 °C and 22 °C for 5-mo storage. There was an apparent increase in total phenolics. Increased extraction efficiency and/or depolymerization of high-molecular-weight phenolics are possible explanations for this increment. Similar to the anthocyanins, approximately 50% of the polyphenolics leached into the syrup with canning. HPLC showed that caffeic acid was formed in cherries (0.4 mg/100 g) and in syrup (0.4 to 0.5 mg/100 g) during canning (data not shown). It is most likely formed from hydrolysis of chlorogenic and/or neochlorogenic acids. Caffeic acid was included in the measurement of hydroxycinnamates. Hydroxycinnamates and epicatechin decreased significantly after 5-mo storage at both 2 °C and 22 °C. The degradation of epicatechin increased significantly at 22 °C. Flavanol glycosides were more stable showing a significant decrease at 2 °C and an apparent increase at 22 °C. There was evidence for an approximate 2× increase of caffeic acid in cherries and syrup after 5 mo of storage at 22 °C (data not shown).

Changes in anthocyanins and polyphenolics during brining

The anthocyanin composition of fresh Bing and Royal Ann cherries is compared with the brined fruit and spent brine after 1 y of storage at 22 °C in Table 7. The anthocyanin pigments become colorless after reactions with SO₂ to form the sulfonic acid addition product. The anthocyanins can be regenerated by treatment with strong acid. Royal Ann cherries have been preferred by the brining industry; however, Bing and other highly pigmented varieties are increasingly being used for brining because of their increased availability. After brining, the cherry fruit itself contained little anthocyanin pigment, but 42% of the anthocyanins from Bing cherry brine could be recovered. Spent cherry brine can be a potential source of anthocyanins. Disposal of spent

Table 6—Total phenolic content, hydroxycinnamates, epicatechin, flavonol glycosides, ORAC, and FRAP in fresh Bing, canned cherries, and their syrup stored at 2 °C and 22 °C for 0 and 5 mo

<table>
<thead>
<tr>
<th>Samples</th>
<th>Portion</th>
<th>Temp (°C)</th>
<th>Time (mo)</th>
<th>TP (mg chlorogenic acid/100 g)</th>
<th>Hydroxycinnamates (mg chlorogenic acid/100 g)</th>
<th>Epicatechin (mg epicatechin/100 g)</th>
<th>Flavonol glycosides (mg rutin/100 g)</th>
<th>ORAC (μmol Trolox equivalent/100 g fw)</th>
<th>FRAP (mole Trolox equivalent/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh cherries</td>
<td>—</td>
<td>2</td>
<td>0</td>
<td>194</td>
<td>70.3 ± 3.42B</td>
<td>11.3 ± 0.82A</td>
<td>6.41 ± 0.09B</td>
<td>1312</td>
<td>1432</td>
</tr>
<tr>
<td>Canned cherries</td>
<td>—</td>
<td>2</td>
<td>0</td>
<td>259</td>
<td>87.7 ± 0.81Aa</td>
<td>10.0 ± 1.14Aay</td>
<td>8.62 ± 0.19Aa</td>
<td>1742</td>
<td>1997</td>
</tr>
<tr>
<td>Cherries</td>
<td>2</td>
<td>0</td>
<td>117</td>
<td>42.9 ± 0.55</td>
<td>5.58 ± 0.90</td>
<td>4.40 ± 0.18</td>
<td>892</td>
<td>959</td>
<td></td>
</tr>
<tr>
<td>Syrup</td>
<td>2</td>
<td>0</td>
<td>141</td>
<td>44.8 ± 0.51</td>
<td>4.43 ± 0.23</td>
<td>4.22 ± 0.29</td>
<td>851</td>
<td>1038</td>
<td></td>
</tr>
<tr>
<td>Canned cherries</td>
<td>—</td>
<td>2</td>
<td>5</td>
<td>235</td>
<td>80.4 ± 1.12b</td>
<td>7.99 ± 0.33ax</td>
<td>7.29 ± 0.51b</td>
<td>2957</td>
<td>2059</td>
</tr>
<tr>
<td>Cherries</td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>127</td>
<td>41.0 ± 1.71</td>
<td>4.29 ± 0.28</td>
<td>3.93 ± 0.28</td>
<td>1418</td>
<td>1007</td>
</tr>
<tr>
<td>Syrup</td>
<td>2</td>
<td>5</td>
<td>108</td>
<td>39.4 ± 1.21</td>
<td>3.70 ± 0.09</td>
<td>3.37 ± 0.25</td>
<td>1538</td>
<td>1052</td>
<td></td>
</tr>
<tr>
<td>Canned cherries</td>
<td>22</td>
<td>0</td>
<td>223</td>
<td>86.1 ± 3.19Aa</td>
<td>8.58 ± 0.29Bby</td>
<td>6.95 ± 0.71Bb</td>
<td>1845</td>
<td>1855</td>
<td></td>
</tr>
<tr>
<td>Cherries</td>
<td>22</td>
<td>0</td>
<td>113</td>
<td>41.9 ± 2.18</td>
<td>4.16 ± 0.35</td>
<td>4.36 ± 0.41</td>
<td>886</td>
<td>879</td>
<td></td>
</tr>
<tr>
<td>Syrup</td>
<td>22</td>
<td>0</td>
<td>120</td>
<td>44.2 ± 1.14</td>
<td>4.43 ± 0.16</td>
<td>4.28 ± 0.03</td>
<td>947</td>
<td>975</td>
<td></td>
</tr>
<tr>
<td>Canned cherries</td>
<td>—</td>
<td>22</td>
<td>5</td>
<td>231</td>
<td>60.5 ± 1.37c</td>
<td>7.22 ± 0.30bx</td>
<td>7.60 ± 0.03b</td>
<td>2996</td>
<td>1903</td>
</tr>
<tr>
<td>Cherries</td>
<td>22</td>
<td>5</td>
<td>121</td>
<td>39.4 ± 0.64</td>
<td>3.54 ± 0.25</td>
<td>3.96 ± 0.15</td>
<td>1276</td>
<td>898</td>
<td></td>
</tr>
<tr>
<td>Syrup</td>
<td>22</td>
<td>5</td>
<td>110</td>
<td>21.1 ± 0.73</td>
<td>3.69 ± 0.08</td>
<td>3.64 ± 0.20</td>
<td>1620</td>
<td>1002</td>
<td></td>
</tr>
</tbody>
</table>

Table 7—Relative anthocyanin composition of fresh cherries, brined cherries, and their spent brine solution

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>ACN (mg/100 g)</th>
<th>Unknown</th>
<th>Cyanidin-3-glucoside</th>
<th>Cyanidin-3-rutinoside</th>
<th>Pelargonidin-3-glucoside</th>
<th>Pelargonidin-3-rutinoside</th>
<th>Peonidin-3-glucoside</th>
<th>Peonidin-3-rutinoside</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bing</td>
<td>26.1</td>
<td>0.63</td>
<td>8.05 ± 0.08</td>
<td>95.2 ± 0.18</td>
<td>0.56 ± 0.03</td>
<td>0.92 ± 0.02</td>
<td>nd</td>
<td>8.11 ± 0.17</td>
</tr>
<tr>
<td>Brined cherries</td>
<td>0.5</td>
<td>0.1</td>
<td>6.82 ± 0.44*</td>
<td>88.3 ± 0.22*</td>
<td>nd*</td>
<td>nd*</td>
<td>nd</td>
<td>4.89 ± 0.27*</td>
</tr>
<tr>
<td>Spent brine solution</td>
<td>11.1</td>
<td>0.63</td>
<td>7.22 ± 0.08*</td>
<td>84.8 ± 0.12*</td>
<td>nd*</td>
<td>0.90 ± 0.01</td>
<td>0.56 ± 0.01*</td>
<td>4.64 ± 0.08*</td>
</tr>
<tr>
<td>Royal Ann</td>
<td>Fresh</td>
<td>0.63</td>
<td>88.1 ± 1.45</td>
<td>11.9 ± 1.45</td>
<td>nd*</td>
<td>nd*</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Brined cherries</td>
<td>0.1</td>
<td>0.1</td>
<td>88.1 ± 1.45</td>
<td>11.9 ± 1.45</td>
<td>nd*</td>
<td>nd*</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Spent brine solution</td>
<td>0.1</td>
<td>0.1</td>
<td>72.6 ± 0.27*</td>
<td>72.6 ± 0.27*</td>
<td>nd*</td>
<td>nd*</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

Notes:
- ACN = anthocyanin content (mg cyanidin-3-glucoside/100 g fresh weight) (Chaovanalikit and Wrolstad 2003). Each value in table is mean ± standard deviation (n = 2). * means a significant difference from fresh cherries at α = 0.05; nd = not detected. Each cultivar was analyzed separately.
brine is an economic and environmental issue. With respect to individual anthocyanins, the most striking finding is the presence of unknown anthocyanins in spent Bing cherry brine. This pigment was not present in the fresh Bing cherries and must have been formed during brining. The hydrolysis of cyanidin-3-rutinoside to cyanidin-3-glucoside occurred during brining. Francis (1972) reported that cyanidin-3-glucoside was produced during acid hydrolysis of cyanidin-3-rutinoside. Peonidin-3-glucoside formed during brining of Bing cherries, undoubtedly from partial hydrolysis of peonidin-3-rutinoside, which decreased significantly.

The HPLC polyphenolic profiles for fresh Royal Ann are shown in Figure 4. No polyphenolics were detected in the brined fruits. In addition to leaching into the brine during storage, there were losses during the extensive water washing to remove residual SO₂. Most of the identified hydroxycinnamates or flavonol glycosides disappeared in the spent brine. There were early eluting peaks in the spent brine, indicating that they were highly polar. Sulfation of polyphenolics would increase polarity. Virtually none of the peaks were identified. Their UV-visible spectra were similar to those of epicatechin and procyanidins, which have no unsaturation in the C-ring.

Relevance of anthocyanin and polyphenolic composition to antioxidant properties

In an earlier publication (Choavanakit and Wrolstad 2003), we reported the antioxidant properties for these cherry extracts as measured by oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP). One purpose of this investigation was to see if polyphenolic composition could provide some insight for the antioxidant level found in the different extracts. Table 2, 4, and 6 list ORAC and FRAP values from the previous investigation. Total phenolics was highly correlated with ORAC ($r = 0.97$) and FRAP ($r = 0.95$), whereas total anthocyanins showed a low correlation with both ORAC ($r = 0.61$) and FRAP ($r = 0.60$). This is to be expected because flesh and pits of some cherry cultivars contained no anthocyanins. Montmorency skins exhibited the highest antioxidant activities and also had the highest levels of total phenolics, flavonol glycosides, and flavanols (Table 2). Although Bing skins had the highest anthocyanin content, their total polyphenolic levels and antioxidant properties were considerably lower than Royal Ann’s. Royal Ann skins were much higher in hydroxycinnamates, epicatechin, flavonol glycosides, and procyanidins, the latter not being detected in Bing skins. ORAC and FRAP values showed the following correlations with individual polyphenolic content: epicatechin ($r = 0.97$, 0.98), total flavonol glycosides ($r = 0.95$, 0.95), procyanidins ($r = 0.81$, 0.88), and total hydroxycinnamates ($r = 0.78$, 0.64). ORAC and FRAP were highly correlated ($r = 0.98$).

Roberds and others (1999) reviewed the ORAC activity of polyphenolic compounds as follows: quercetin > kaempferol > epicatechin > caffeic acid > P-coumaric acid > pelargonidin > quercetin-3-rutinoside. Kähkönen and Heinonen (2003) reported that the antioxidant power of anthocyanins depended on the type of anthocyanin and the glycosylation pattern. Wang and others (1997a) reported the ORAC activity of anthocyanin, in decreasing order: cyanidin-3-glucoside, cyanidin, peonidin-3-glucoside = delphinidin, peonidin, cyanidin-3,5-diglucoside, pelargonidin-3-glucoside, pelargonidin, and pelargonidin-3,5-diglucoside.

Polymerization of flavan-3-ols from monomer to trimer can increase the antioxidant activity in aqueous phases (Plumb and others 1998). It has been recognized that synergistic effects between polyphenolics and other phytochemicals can also enhance antioxidant activity (Saucier and Waterhouse 1999; Kähkönen and others 2001).

Total phenolics declined during frozen storage of Bing cherries at –23 °C, and both ORAC and FRAP also decreased (Table 4). We have no good explanation, however, for the marked increase in both ORAC and FRAP for Bing cherries stored at –70 °C. Changes of anthocyanin and polyphenolic composition may be a possible explanation because the structure is associated with the antioxidant activity. Although cannning resulted in increased levels of total phenolics, both ORAC and FRAP increased by even greater orders of magnitude (Table 6). FRAP and particularly ORAC increased during storage at both temperatures. One explanation could be the formation of Maillard reaction products from sugars and amino acids. Several workers have demonstrated that Maillard reaction products can increase antioxidant activities (Anese and others 1999; Anthony and others 2000; Mastrocol and Munari 2000).

Conclusions

Although the qualitative anthocyanin and polyphenolic composition for the different cherry cultivars is similar, there are major quantitative differences. Sour cherries, not surprisingly, show more compositional differences than the 3 sweet cherry cultivars. All sweet cherries were high in hydroxycinnamates, with Rainier having the lowest amounts. Sour cherries were much higher in procyanidins, flavonol glycosides, and flavanols than sweet cherries. Bing cherries were highest in anthocyanins and the only cultivar with pigmentation in the flesh; however, it was the only cultivar in which procyanidins were not detected in skins and flesh. Flavonol glycosides were almost exclusively present in the skins. Both skins and pits may be potential sources for polyphenolics and anthocyanins. Because Montmorency is highest in antioxidant properties and Bing and Royal Ann are comparable, it would appear that procyanidins might play a particularly important antioxidant role.

Anthocyanins and polyphenolics underwent pronounced degradation during frozen storage at –23 °C but were relatively stable at –70 °C. Destruction is believed to be because of native enzymes, particularly polyphenoloxidase. During cannning, approximately half of the anthocyanins and polyphenolics were redistributed to the syrup up with no apparent loss of total anthocyanins. Anthocyanin-rutinosides underwent partial hydrolysis during frozen storage and canned storage. Anthocyanins and polyphenolics were nearly completely removed from the fruits during brining and washing operations. Nearly half of the Bing anthocyanins could be recovered from the spent brine. Hydrolysis of anthocyanin-rutinosides to glucosides occurred during brining with the formation of unknown anthocyanins. Hydroxycinnamates and epicatechin were more labile than flavonol glycosides during processing and storage. The polyphenolic profile of spent cherry brine was extremely different from the original fruits, with numerous unidentified polyphenolic derivatives being formed.

Acknowledgments

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