AN ABSTRACT OF THE DISSERTATION OF

Arusa Chaovanalikit for the degree of Doctor of Philosophy in Food Science and Technology presented on June 3, 2003.
Title: Cherry Phytochemicals

Abstract approved:

Ronald E. Wrolstad

The distribution of anthocyanin pigments and polyphenolics of sweet (*Prunus avium*) and sour cherries (*Prunus cerasus*) were determined by Ultraviolet-Visible (UV-Visible) spectrophotometry and High Performance Liquid Chromatography with photodiode array detector (HPLC–DAD). Their antioxidant properties were determined by Oxygen Radical Absorbance Capacity (ORAC) and Ferric Reducing Antioxidant Power (FRAP). The effect of frozen storage, canning, and brining on those properties was measured.

Experiments were conducted on three sweet cherry cultivars; Bing, Rainier, Royal Ann and one sour cherry cultivar; Montmorency. Cherries were separated into skins, flesh, pits, and pitted cherries for subsequent analyses. Bing had the highest anthocyanin pigments (60.6 mg/100g fw) while Montmorency had both the highest total phenolic content (5.6 mg GAE/g fw) and the highest antioxidant activities (ORAC 51.02 μmoles Trolox equivalent (TE) /g fw, FRAP 47.96 μmoles

TE/g fw). Hydroxycinnamates predominated in sweet cherries (70-80%) while flavanols were the major class of polyphenolics in sour cherries (70%). The major anthocyanins in sweet and sour cherries were cyanidin-3-rutinoside and cyanidin-3-glucosylrutinoside, respectively. Skins contained the highest amount of anthocyanins, polyphenolics, and antioxidant activities. Anthocyanins and flavonol glycosides predominated in cherry skins. Bing cherries were different from the others in that it had substantial anthocyanins in flesh and pits. The proportion of flavanols increased from skins to pits.

Pitted Bing cherries were frozen and stored at -23 and –70 °C for 3 and 6 months. Pitted Bing cherries were also canned in light syrup and stored at 2 and 22 °C for 5 months. Both Bing and Royal Ann cherries were brined in bisulfite for one year. In all processing experiments, polyphenolics were more stable than anthocyanins. Degradation of hydroxycinnamates occurred during frozen storage and canning while flavonol glycosides were relatively stable. With both canning and brining, anthocyanins and polyphenolics leached into syrup and brine. With brining, hydroxycinnamates and flavonol glycosides disappeared, and unidentified compounds with UV-Visible spectra similar to flavanols were formed. Unidentified compounds possessed antioxidant activity.

Cherry skins are high in anthocyanins, polyphenolics and antioxidant properties. Cherry pits and spent brine solution may be a potential source for natural colorants, nutraceuticals, and natural antioxidants.

Cherry Phytochemicals

by

Arusa Chaovanalikit

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APPROVED:

Major Professor, representing Food Science and Technology

Head of Department of Food Science and Technology

Dean of the Graduate School

I understand that my dissertation will become part of permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request

Arusa Chaovanalikit, Author

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CONTRIBUTIONS OF AUTHOR

Dr. Ronald E. Wrolstad was involved in the design, analysis, and writing of each manuscript.

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CHERRY PHYTOCHEMICALS

CHAPTER I.INTRODUCTION

Cherries are a major economic crop in the United States. Sweet cherries are primarily grown in Washington, Oregon, and California while Michigan is the major growing area of sour cherries. Previous studies suggested that the health benefits of cherries are due to their antioxidant and anti-inflammatory activities *(117, 141, 144)*. Polyphenolics, which are plant secondary metabolites, are believed to provide those benefits *(22, 26, 48, 53, 60, 66, 67, 68)*. Most of the previous research has been conducted on sour cherries. The subject of this investigation is to complete the comparative study on the polyphenolics and their benefits between sweet and sour cherries.

Several polyphenolics in sweet and sour cherries have been identified. Less attention has been given to the polyphenolics of light-colored sweet cherries, such as Royal Ann and Rainier. There is no previous study comparing the distribution of polyphenolics in cherries. Cherry skins and pits are cherry processing wastes. Investigation on the cherry polyphenolic distribution may suggest ways to utilize and add value to waste, which may be a potential source for natural colorants, nutraceuticals, and natural antioxidants.

In the United States, about 40 % of US sweet cherry production is processed into frozen, canned, and brined cherries for subsequent processing into

maraschino cherries *(1)*. There is intense interest in the possible health benefits of fruits. More complete information is needed on the amounts of polyphenolics in fresh and processed cherries and their antioxidant properties. As cherries contain beneficial compounds such as anthocyanins and polyphenolics, cherry products may find increased usage for ingredients for functional foods.

The objectives of this study are to evaluate the distribution of polyphenolics in sweet and sour cherries, to determine and compare the antioxidant activity of sweet and sour cherries, and to investigate the effect of processing on polyphenolic composition and antioxidant activity in cherries.

CHAPTER II. LITERATURE REVIEW

CHERRIES

Cherries are taxonomically classified in the genus *Prunus*, which is part of *Rosaceae* family. Cherries are believed to have originated around the Caspian and Black seas. From there, cherry production expanded throughout the world to the United States, Germany, Yugoslavia, and Italy (*27*). During 1997 to 2001, the United States produced about 370,000 tons of cherries (sweet cherries >50% and sour cherries <50%) and is considered as the second largest cherry producer in the world (*1*).

Cherries are categorized into two major types: sweet cherries (*Prunus avium* L.) and sour or tart cherries (*Prunus cerasus* L.). In the United States, sweet cherries are primarily grown in the Pacific Northwestern States of Washington, Oregon, and California, whereas sour cherries are mainly produced in the Great Lakes area, predominantly in Michigan.

In the UK, the USA, and France, sweet cherries are usually characterized into two major groups based on the flesh firmness: 1) the Hearts, Geans, or Guignes and 2) the Bigarreaux (*10*). The Hearts, Geans, or Guignes are the soft-fleshed cherries such as Black Tartarian while the Bigarreaux are the hard-fleshed cherries, for example, Napoleon or Royal Ann and Bing (*10, 107*). In 2001, the Pacific Northwest produced about 87% of the US sweet cherry production (*72*). Bings are the most important sweet cherries in the United States due to the strong

demand in many parts of the world (*107*). Royal Ann cultivar is widely used for processing particularly in brining for maraschino cherry production.

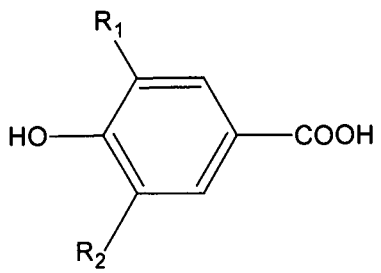
Sour cherries are generally categorized into two groups: the Amarelles and the Morellos. The Amarelles are the uncolored fleshed cherries with uncolored juice, for example, Montmorency, which is used exclusively for cherry pie filling (*44*). The Morellos are red to dark red flesh and juice. In Europe, the Morello cultivar is produced for a wide range of cherry products, for instance, juices and jams. Several sour cherries are grown in North America ranging from the light red (early Richmond) to the medium red skinned (Montmorency) and the late dark red (English Morello) (*27*). Michigan produced about 80% of the USA sour cherry production in 2001 (*72*). More than 95 % of the sour cherries cultivated in the United States is Montmorency (*103*).

ANTHOCYANINS AND POLYPHENOLICS IN CHERRIES

Polyphenolics are secondary plant metabolites derived from the shikimate pathway and phenylpropanoid metabolism (*25*). They are widely distributed in several parts of plants such as leaves, barks, seeds and fruits. They play an important role in defense mechanisms against infection and injury, as well as color and flavor (*84*). Anthocyanins are responsible for red color in cherry skins.

Dietary polyphenolics are classified into 3 groups: phenolic acids, flavonoids, and tannins (*53*). Phenolic acids include hydroxybenzoic acid derivatives such as *p*-hydroxybenzoic acid and protocatechuic acid (Figure 1.1) and

Figure 1.1: Chemical structures of hydroxybenzoic acid derivatives

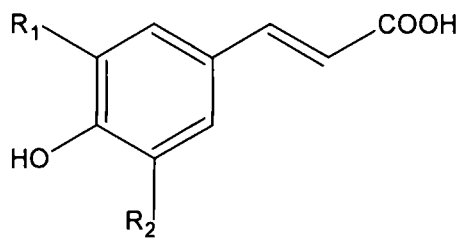


Hydroxybenzoic acids	R1	R2
p-hydroxybenzoic acid	H	H
Protocatechuic acid	OH	H
Vanillic acid	OCH3	H
Gallic acid	OH	OH
Syringic acid	OCH3	OCH3

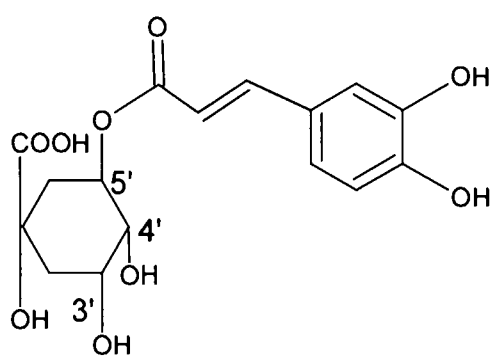
hydroxycinnamic acid derivatives or hydroxycinnamates such as chlorogenic acid and *p*-coumaroylquinic acid (Figure 1.2) (*53, 84*).

Dietary flavonoids include anthocyanins such as cyanidin-3-glucoside (Figure 1.3), flavanols such as catechin and epicatechin (Figure 1.4), flavones such as luteolin (Figure 1.5), flavanones such as hesperitin and naringenin (Figure 1.6), and flavonols such as quercetin and kaempferol (Figure 1.7) (*84, 106*). Tannins are high molecular weight compounds, which are divided into condensed tannins including oligomeric and polymeric procyanidins (Figure1.8) and hydrolyzable tannins such as gallotannin and ellagitannin (*52*).

Figure 1.2: Chemical structures of hydroxycinnamic acid and its derivatives



Hydroxycinnamic acids	R1	R2
p-Coumaric acid	H	H
Caffeic acid	OH	H
Ferulic acid	OCH3	H
Sinapic acid	OCH3	OCH3

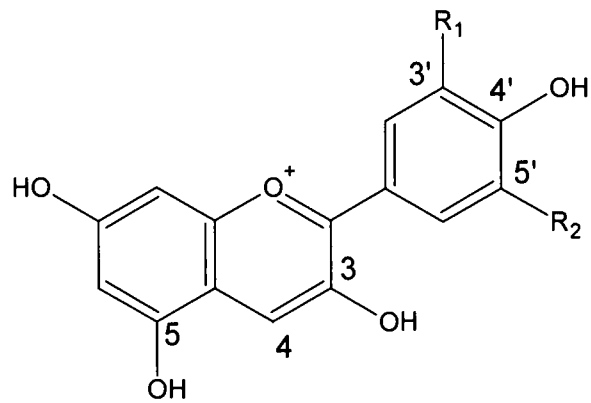


5'-Caffeoylquinic acid = Chlorogenic acid

4'-Caffeoylquinic acid = Cryptochlorogenic acid

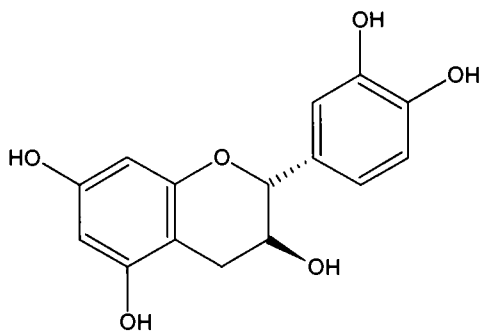
3'-Caffeoylquinic acid = Neochlorogenic acid

Figure 1.3: Chemical structures of anthocyanidins

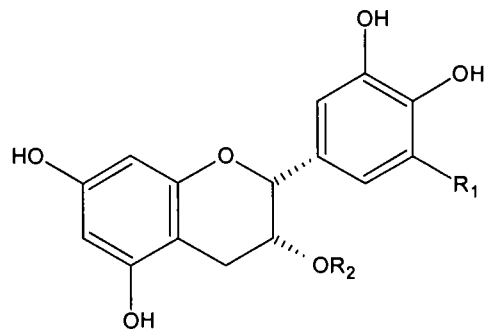


Anthocyanidins	R1	R2
Pelargonidin	H	H
Cyanidin	H	OH
Delphinidin	OH	OH
Peonidin	OCH_3	H
Petunidin	OCH_3	OH
Malvidin	OCH_3	OCH_3

Figure 1.4: Chemical structures of flavanols



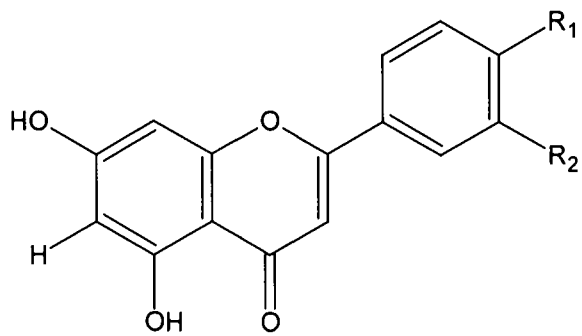
(A)



(B)

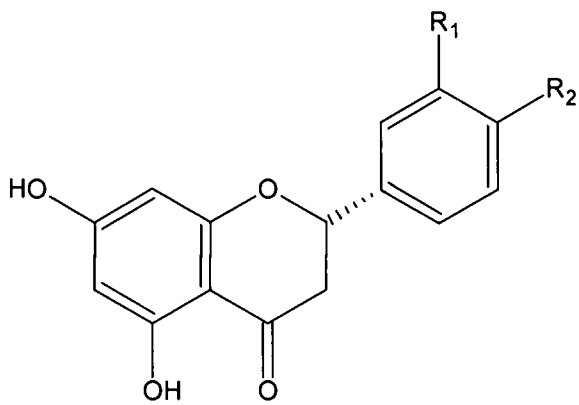
Flavanols	R1	R2
(+) Catechin(A)	-	-
(-)- Epicatechin	H	H
(-)-Epigallocatechin	OH	H

Figure 1.5: Chemical structures of flavones



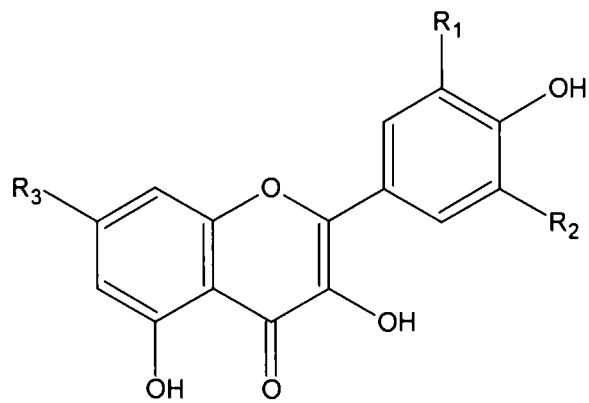
Flavone	R1	R2
Apigenin	OH	H
Luteolin	OH	OH

Figure 1.6: Chemical structures of flavanones



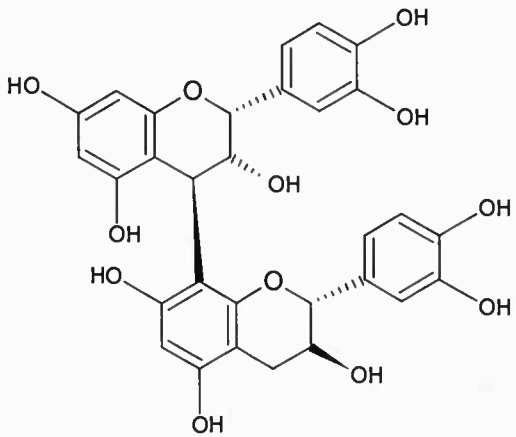
Flavanone	R1	R2
Naringenin	H	OH
Hesperetin	OH	OCH_3

Figure 1.7: Chemical structures of flavonols

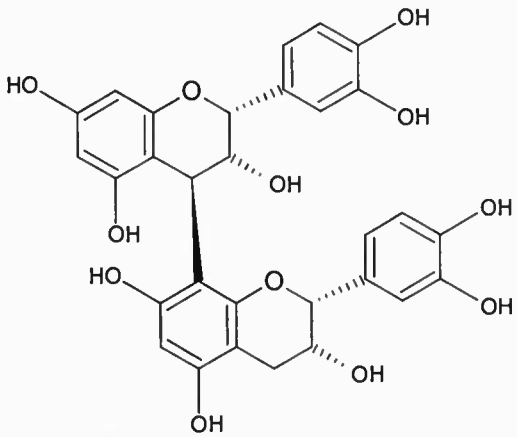


Flavonols	R1	R2	R3
Kaempferol	H	H	OH
Quercetin	OH	H	OH
Myricetin	OH	OH	OH
Rhamnetin	OH	H	OCH_3
Isorhamnetin	OCH_3	H	OH

Figure 1.8: Chemical structures of procyanidins



(A) Procyanidin B1



(B) Procyanidin B2

The identification and quantification of polyphenolics in sweet and sour cherries are reviewed and reported (*22, 26, 33, 35, 40, 57, 59, 85, 91, 92, 102, 103, 104*). Major polyphenolics in cherries are hydroxycinnamates, anthocyanins, flavonols and flavonol glycosides, and flavanols.

Sweet cherries (Prunus avium)

Lynn and Luh (*57*) identified the major anthocyanins in Bing cherries as cyanidin-3-rutinoside and cyanidin-3-glucoside with the minors as peonidin-3-glucoside and peonidin-3-rutinoside. These results were confirmed by Gao and Mazza (*35*) and Mozetič et al (*69*). Gao and Mazza (*35*) and Mozetič et al. (*69*) additionally identified the presence of pelargonidin-3-rutinoside in Bings and other sweet cherries. Cyanidin-3-rutinoside and cyanidin-3-glucoside are reported in the Windsor variety (*55*) while peonidin-3-glucoside is not present in the Bargioni cultivar (*79*). Table 1.1 illustrated the anthocyanin content in sweet cherries.

Table 1.1: Anthocyanin content in sweet and sour cherries

Cultivar	Growing region	Method	Anthocyanins [a]	N	Ref.
Sweet cherries					
	MI, USA	HPLC	25.95		(*88*)
Bing	BC, Canada	HPLC	224.67	5	(*35*)
Bing	Nova Gorica Slovenia	HPLC, Spec.	28.19, 28.12	3	(*69*)
Lambert	BC, Canada	HPLC	198.11	5	(*35*)
Lambert	Nova Gorica Slovenia	HPLC, Spec.	29.28, 28.23	3	(*69*)
Napoleon	Nova Gorica Slovenia	HPLC, Spec.	33.71, 30.94	3	(*69*)
Petrovka	Nova Gorica Slovenia	HPLC, Spec.	62.13, 63.42	3	(*69*)
Sam	BC, Canada	HPLC	227.04	5	(*35*)
Stella	BC, Canada	HPLC	154.19	5	(*35*)
Stella Compact	Nova Gorica Slovenia	HPLC, Spec.	38.91, 35.24	3	(*69*)
Summit	BC, Canada	HPLC	81.95	5	(*35*)
Sylvia	BC, Canada	HPLC	245.21	5	(*35*)
Van	BC, Canada	HPLC	150.76	5	(*35*)
Light-colored cherries	BC, Canada	HPLC	2-41		(*35*)
Sour cherries					
Balaton	MI, USA	HPLC	23.6-37.5		(*88, 102*)
Balaton	MI, USA	HPLC	50.1-52.6	5	(*23*)
Montmorency	MI, USA	HPLC	7.52-16.45		(*88, 102*)

[a]Anthocyanin content was measured spectrophotometrically as mg cyanidin-3-glucoside/100g fresh weight (fw) except for Ref (23),as mg cyanidin-3-rutinoside/100g fw, and for Ref (88, 102) where anthocyanin content was measured gravimetrically; HPLC = High performance liquid chromatography; Spec = spectrophotometry method; N = number of samples;
Ref = reference.

Sweet cherries are rich sources of polyphenolics. The dominant polyphenolics in sweet cherries are hydroxycinnamates, flavanols and flavonol glycosides (*33*). Neochlorogenic acid or 3'-caffeoylquinic acid and 3'*p*-coumaroylquinic acid are predominant in sweet cherries (*35, 64, 69*). Friedrich and Lee (*32, 33*) reported that caffeoyltartatric acid or caftaric acid is a major phenolic in sweet cherries (unpublished data). The presence of several minor hydroxycinnamates and other phenolic acids were summarized in a review by Machiex et al. (*59*) as follows: chlorogenic acid, 4'-caffeoylquinic acid, 4'-*p*-coumaroylquinic acid, 5'-*p*-coumaroylquinic acid, 3'-feruloylquinic acid, 5'-feruloylquinic acid, di-caffeoylquinic acid, *p*-hydroxybenzoic acid, vanillic acid, *p*-hydroxybenzoic acid glucoside, protocatechuic acid glucoside, and vanillic glucoside (*59*). Table 1.2 illustrates the hydroxycinnamate levels in sweet cherries. The ratio of neochlorogenic acid to 3'-*p*-coumaroylquinic acid varies widely among the cherry cultivars (*35, 69*). Bings contained 60% of neochlorogenic acid and 40% of 3'-*p*-coumaroylquinic acid while Burlats contained 16.5% of neochlorogenic acid and 75% of 3'-*p*-coumaroylquinic acid (*35*).

Flavan-3-ols, catechin and epicatechin, have been identified in sweet cherries (*5, 32, 33, 59*) (Table 1.3). Burlat sweet cherries contained 2.52-2.63 mg /100g (*38*). The epicatechin and catechin content in sweet cherries are reported as 2.02- 9.53 and 0-2.2 mg/100g, respectively (*5, 32, 33*).

Table 1.2: Total phenolics and hydroxycinnamate content in sweet and sour cherries

Cultivar	Growning region	Total phenolics[a]	Neochlorogenic acid	Cafffeoyltartaric acid	3'-*p*-coumaroylquinic acid	4'-*p*-coumaroylquinic acid	N	Ref
Sweet cherries								
	NY, USA	-	0.96[c]	13.02	11.66	nd	-	(*33*)
Bing	BC, Canada	-	128.16	-	42.67	-	5	(*35*)
Bing	Nova Gorica, Slovenia	97.38	27.12	-	8.04	-	3	(*69*)
Lambert	BC Canada	-	118.89	-	46.99	-	5	(*35*)
Lambert	Nova Gorica, Slovenia	117.22	35.5	-	8.54	-	3	(*69*)
Napoleon	Nova Gorica, Slovenia	144.02	19.46	-	50.65	-	3	(*69*)
Petrovka	Nova Gorica, Slovenia	196.98	53.05	-	16.42	-	3	(*69*)
Sam	BC, Canada	-	38.40	-	131.45	-	5	(*35*)
Stella	BC, Canada	-	92.92	-	32.54	-	5	(*35*)
Stella	Nova Gorica, Slovenia	120.98	30.24	-	7.52	-	3	(*69*)
Compact Summit	BC, Canada	-	28.96	-	84	-	5	(*35*)
Sylvia	BC, Canada	-	34.21	-	76.34	-	5	(*35*)
Van	BC, Canada	-	87.54	-	23.02	-	5	(*35*)
Light-colored cherries	BC, Canada	-	49.53-66.86	-	94.24-126.08	-	5	(*35*)
Sour cherries								
	NY, USA	48-99	7.61	9.37	2.78	8.66		(*33*)

[a]Total phenolics was measured by the Folin-Ciocalteu Method as mg gallic acid/100 g fw while others was measured as mg/100 g fw

Flavonols and flavonol glycosides are another important class of sweet cherry polyphenolics. The rutinosides and glucosides of quercetin and kaempferol are major compounds with minor amount of quercetin-3-galactoside, quercetin-3-rhamnoside, kaempferol galactoside, kaempferol-3-rutinosyl-4'-diglucoside, quercetin-3-rutinosyl-4'-diglucoside, and quercetin-3-rutinosyl-7,3'-diglucoside (*39, 40*). Melin et al. (*65*) additionally identified the isorhamnetin-3-rutinoside, kaempferol-7-glucoside (tentative), and kaempferol-3-(2^G-glucosylrutinoside) (tentative) in Bigarreau Napoleon cherries. Bing and Burlat sweet cherries contain 1-1.8 and 1.5-2.3 mg/100g while yellow sweet cherries contained 0.3-0.9 mg/100g (*32, 38*).

Sour cherries (Prunus cerasus)

The anthocyanin content of sour cherries is illustrated in Table 1.1. Dekazos (*26*) identified the major anthocyanins in Montmorency as cyanidin-3-glucosylrutinoside and cyanidin-3-rutinoside with two minor anthocyanins: cyanidin-3-glucoside and peonidin-3-rutinoside. The results were confirmed by Shrikhande and Francis (*91*), Chandra et al. (*23*), and Hong and Wrolstad (*43*). HPLC-MS confirmed the presence of the four former anthocyanins as well as the presence of cyanidin-3-arabinosyl-rutinoside (*23*). Cyanidin-3-sophoroside (*22, 43*), cyanidin-3-gentibioside (*86, 94*), and cyanidin-3-(2^G-xylosylrutinoside (*28, 91*) were identified in sour cherries.

Sour cherries contain polyphenolics similar to those of sweet cherries. Schaller and Von Elbe (*85*) reported the presence of six isomers of caffeoylquinic acid, four isomers of *p*-coumaroylquinic acid, caffeic acid, and *p*-coumaric acid. Macheix et al. (*59*) summarized the major sour cherry phenolics from the literature with neochlorogenic acid as the major phenolic and in addition, 4'-caffeoylquinic acid, chlorogenic acid, 3'-*p*-coumaroylquinic acid, 4'-*p*-coumaroylquinic acid, 5'-*p*-coumaroylquinic acid, 3'-feruloylquinic acid, 5'-feruloylquinic acid, di-caffeoylquinic acid, vanillic acid, and *p*-coumaric glucose. The presence of neochlorogenic acid, chlorogenic acid, 3'-*p*-coumaroylquinic acid and 4'-*p*-coumaroylquinic acid were confirmed by Friedrich and Lee (*32, 33*) and Wang et al. (*103*). Table 1.2 summarizes the hydroxycinnamic content in sour cherries.

The presence of epicatechin, catechin, gallocatechin, and epigallocatechin in sour cherries were reviewed by Macheix et al. (*59*). Epicatechin was reported as 4.8 mg/100 g in sour cherries (*33*). Friedrich and Lee (*32, 33*) reported that procyanidin B-2 accounted for 33% of all non anthocyanin polyphenolics in sour cherries.

Schaller and Von Elbe (*85*) identified the glucoside and rutinoside of kaempferol in sour cherries. The results were confirmed by Shrikhande and Francis (*92*) and Macheix et al. (*59*) along with other flavonol glycosides: quercetin-3-glucoside, quercetin-3-galactoside, quercetin-3-rutinoside, quercetin-4'-glucoside (tentative), quercetin-3-glucosyl-7-diglucoside (tentative), quercetin-3-galactosyl-7-diglucoside (tentative), quercetin-3-rutinosyl-4'-diglucoside,

kaempferol –3-galactoside, kaempferol-4'-glucoside (tentative), kaempferol-3-galactosyl-7-diglucoside (tentative), kaempferol-3-rutinosyl-4'-diglucoside, and kaempferol-3-rhamnosyl-4'-diglucoside (tentative). Wang et al. (*103*) reported the presence of quercetin-3-rhamnoside, and rhamnazin-6'-rutinoside with the presence of flavanones such as naringenin and isoflavones such as genistein and genistein-7-glucoside.

HEALTH BENEFITS OF POLYPHENOLICS

Several epidemiological studies have indicated that high consumption of fruits and vegetables can reduce the risk of oxidative stress diseases, for example, cardiovascular disease, cancer, stroke, arthritis, macular degeneration, Alzheimer's disease, Parkinson's disease, and aging (*3, 51, 58, 77*).

Oxidation is an essential part of human metabolism, which creates free radicals as by products. Free radicals such as superoxide, peroxyl, alkoxyl, hydroxyl, nitric oxide, singlet oxygen, hypochlorous acid, and hydrogen peroxide cause extensive damage to DNA, proteins, and lipids in human (*37*). Antioxidants produced in the body and obtained from food are important to protect the human body against free radicals (*77*). Antioxidant systems in the human body include enzyme defense, non-enzyme defense, and repair antioxidants. Examples of enzyme defenses are superoxide dismutase, glutathione peroxidase, and catalase, and examples of non-enzyme defense are the iron-binding proteins transferrin and ferritin (*51, 77*). Superoxide dismutase is able to convert superoxide to hydrogen

peroxide and catalase, and glutathione peroxidase will convert hydrogen peroxide to oxygen and water (*51*). Transferrin and ferritin chelate free iron or copper salts, catalysts of hydroxyl radical formation (*3*). Despite these defense mechanisms, some free radicals still escape and cause oxidative damage. Several enzymes are able to remove the oxidized protein, e.g. proteases, and repair the oxidized DNA, e.g. glycosylases (*3, 77*).

Due to the incomplete efficiency of the defenses and repair mechanisms, and physiopathological situations, for example, cigarette, smoking, air pollutants, UV radiation, high polyunsaturated diet and inflammation providing free radical species, the oxidative damages still occur (*77, 95*). It is believed that the consumption of dietary antioxidants from fruits and vegetables may help to reduce the oxidative stress in humans. Cao et al. (*20*) indicated that high consumption of fruits and vegetables increased human plasma antioxidant activity. The relationship between polyphenolics and antioxidant activity in several fruits has been reported and acknowledged as a health benefit from consuming high antioxidant fruits (*8, 9, 16, 38, 49, 54, 66, 67, 68, 77, 84, 89*).

Polyphenolics, for instance, anthocyanins and procyanidins, reduce low-density lipoprotein oxidation considered as an early event in the development of atherosclerosis (*38, 66, 67, 80*). Heinonen et al. (*38*) suggested that antioxidant activity in berries and cherries for low-density lipoprotein was associated with the presence of anthocyanins and the absence of flavonols, whereas for liposome was correlated with hydroxycinnamic acid. Although polyphenolics provide

antioxidant activity, the toxicity of a simple phenolic, protocatechuic acid, at high dose, has been reported. Nakamura et al. (*71*) demonstrated the enhancement of mouse skin tumors with a higher dose of protocatechuic acid at the same time as at lower dose exerted chemoprotective activity in mouse skin. Overdose of protocatechuic acid interfered with the detoxification of toxicants in liver and kidney such as carcinogens (*71*).

ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF CHERRIES

Heinonen et al. (*38*) compared the in vitro antioxidant activities of several different fruits. Sweet cherries (Bing and Burlat cultivars) were ranked in first and third in antioxidant activity to prevent liposome and low-density lipoprotein oxidation. Seeram et al. (*88*) demonstrated that the antioxidant activity of cherries at 125 μg/mL was comparable to the commercial antioxidants, tert-butylhydroquinone, butylated hydroxytoluene and butylated hydroxyanisole, and superior to vitamin E at 10 μM concentration. The use of tart cherry tissues as a natural antioxidant in cooked beef patties has been demonstrated (*17*). Cherry tissue reduces the oxidation in lipid and cholesterol and the formation of heterocyclic aromatic amine in cooked beef patties. The antioxidant and anti-inflammatory properties are associated with anthocyanins in sweet and sour

cherries (*88, 101*). Tables 1.3 and 1.4 summarize the antioxidant and anti-inflammatory activity of sweet and sour cherries by several analytical methods (Table 1.3).

METHODS TO DETERMINE ANTIOXIDANT ACTIVITY

There are numerous methods for evaluating the antioxidant activity in fruits and vegetables such as the Trolox Equivalent Antioxidant Activity assay (TEAC), Diphenyl-1-picrylhydrazyl assay (DDPH), Total Radical-Trapping Antioxidant Parameter assay (TRAP), Oxygen Radical Absorbance Capacity (ORAC) and Ferric Reducing Antioxidant Power (FRAP) which have been studied and reviewed (*6, 18, 34, 38, 49, 75, 81, 84, 105*). ORAC and FRAP have received widespread acceptance for evaluating the antioxidant activity in fruits and vegetables (*4, 14, 20, 68, 89, 98*). Moyer et al. (*68*) showed that ORAC and FRAP were highly correlated to each other and to total phenolics.

Table 1.3: Antioxidant activity in sweet and sour cherries

Reference	Samples	Measurement	Result
Vinson et al. (*97*)	Cherry	The concentration to inhibit LDL+VLDL oxidation 50%	0.10 μM
Karakaya et al.(*50*)	Cherry	Total antioxidant activity (TAA)	1.65 mM
Heinonen et al (*38*)	Sweet cherry extract (10 μM GAE)	Inhibition of LDL oxidation	70-72.7% Inh
	Sweet cherry extract (20 μM GAE)		99.7-99.9% Inh
Heinonen et al (*38*)	Sweet cherry extract (10 μM GAE)	Inhibition hexanal formation of liposome oxidation	92.6-82.9 % Inh
	Sweet cherry extract (20 μM GAE)		97.7-88.8 % Inh
Murcia et al. (*70*)	Sweet cherries var Stark (100 mg)	Inhibition of peroxidation	60.4 % Inb
Murcia et al. (*70*)	Sweet cherries var Stark(100 mg)	Inhibition of deoxyribose damage by hydroxyl radical	96.7 % Inb
Murcia et al. (*70*)	Sweet cherries var Stark(100 mg)	Inhibition of elastase activity by hypochlorous acid	15.5 % Inb
Murcia et al. (*70*)	Sweet cherries var Stark(100 mg)	Hydrogen peroxide scavenging	51 .3% Inb
Wang et al (*104*).	Tart cherry anthocyanins		
	Cyanidin-3-glucosylrutinoside (2 μM)	Inhibition of peroxidation	39% Inb
	Cyanidin-3-rutinoside (2 μM)	Inhibition of peroxidation	70% Inb
	Cyanidin-3-glucoside (2 μM)	Inhibition of peroxidation	75% Inb
	Cyanidin (2 μM)	Inhibition of peroxidation	57% Inb

Concentrations of samples are in the parentheses; GAE= Gallic acid equivalent; % Inb = % Inhibition;
LDL = Low density lipoprotein; VLDL = Very low density lipoprotein

Table 1.4: Anti-inflammatory activity in sweet and sour cherries

Reference	Samples	Measurement	Result
Seeram et al. (*88*)	Sweet cherry anthocyanins at 125 μg/ml	Inhibitory activities of COX-I, II	28.8%, 47.4%
Seeram et al. (*88*)	Balaton tart cherry anthocyanins at 125 μg/ml	Inhibitory activities of COX-I, II	26.6%, 38.3%
Seeram et al. (*88*)	Montmorency tart cherry anthocyanins at 125 μg/ml	Inhibitory activities of COX-I, II	24.9%, 36.6%
Wang et al. (*104*)	Tart cherry anthocyanins		
	Cyanidin-3-glucosylrutinoside	The concentration that exhibit 50% maximal PGHS-I, II activity	Little-No activity
	Cyanidin-3-rutinoside	The concentration that exhibit 50% maximal PGHS-I, II activity	Little-No activity
	Cyanidin-3-glucoside	The concentration that exhibit 50% maximal PGHS-I, II activity	Little-No activity
	Cyanidin	The concentration that exhibit 50% maximal PGHS-I, II activity	90, 60 μM

PGHS-I and –II = Prostaglandin endoperoxide H synthase-I and –II isoenzyme; COX-I and II = Cyclooxygenase –I, II

Principle of Oxygen Radical Absorbance Capacity (ORAC)

Oxygen Radical Absorbance Capacity was initially developed by Cao et al. (*19*). In the ORAC assay, the radical initiator generates radical species such as the peroxyl radicals, which can react with a target molecule probe such as β-phycoerythrin or fluorescein. As a result, a decrease in the fluorescence emission is observed (*19, 75*). In the presence of antioxidants, inhibition of the reactive specie action protects the loss of fluorescence emission indicating the antioxidant capacity against the reactive species. The fluorescence emission is recorded from the time that reaction of antioxidants and radical species occurred every 1-2 minutes over 60-70 minutes. The use of area-under-curve (AUC) technique combines both inhibition time and the inhibition degree of free radical action by antioxidants in a single quantity. This technique allows ORAC to evaluate the antioxidant activity against free radicals (*19, 21, 75, 100*). The antioxidant activity is assessed by comparing the ability of Trolox (the water-soluble vitamin E analogue) to delay the oxidation of peroxyl radicals (*19, 21, 75, 100*).

ORAC has been used to evaluate the antioxidant activity in several fruits and vegetables (*68, 81, 99*). The ORAC values of small berries such as blueberries and blackberries varied from 13 to 146 μmoles Trolox equivalent (TE)/g (*68*). The ORAC value of red plums (25.6 μmoles TE/g) is comparable to strawberries (24.4 μmoles TE/g) and superior to raspberries (18.5 μmoles TE/g), peaches (7.6 μmoles TE/g), pears (5.9 μmoles TE/g), and apples (5.6 μmoles TE/g) (*81*). However, the ORAC values of sweet and sour cherries have not been published.

Principle of Ferric Reducing Antioxidant Power (FRAP)

Benzie and Strain (*12, 13, 14*) have developed the FRAP method by considering the total antioxidant power as total reducing power since the antioxidants reduce the oxidants which can damage substrates. In FRAP, antioxidants reduce ferric tripyridyltriazine (Fe^{III}-TRTZ) complex to a ferrous form, which has an intense blue color at low pH of 3.6. The ferrous form can be monitored by measuring the change of absorbance at 593 nm. The absorbance value is directly related to the reducing power of antioxidants (*13*). Like ORAC, Trolox is used as the standard for comparing the reducing properties among samples. However, ascorbic acid can be used as the standard as well (*36*). Recently, Ou et al. (*75*) suggested several drawback of FRAP assays such as interference, reaction kinetics, and quantitation methods. They also proposed not to use FRAP assay as an indicator of total antioxidant power since FRAP determines the reducing capability based on ferric ion which is irrelevant to antioxidant activity mechanistically and physiologically.

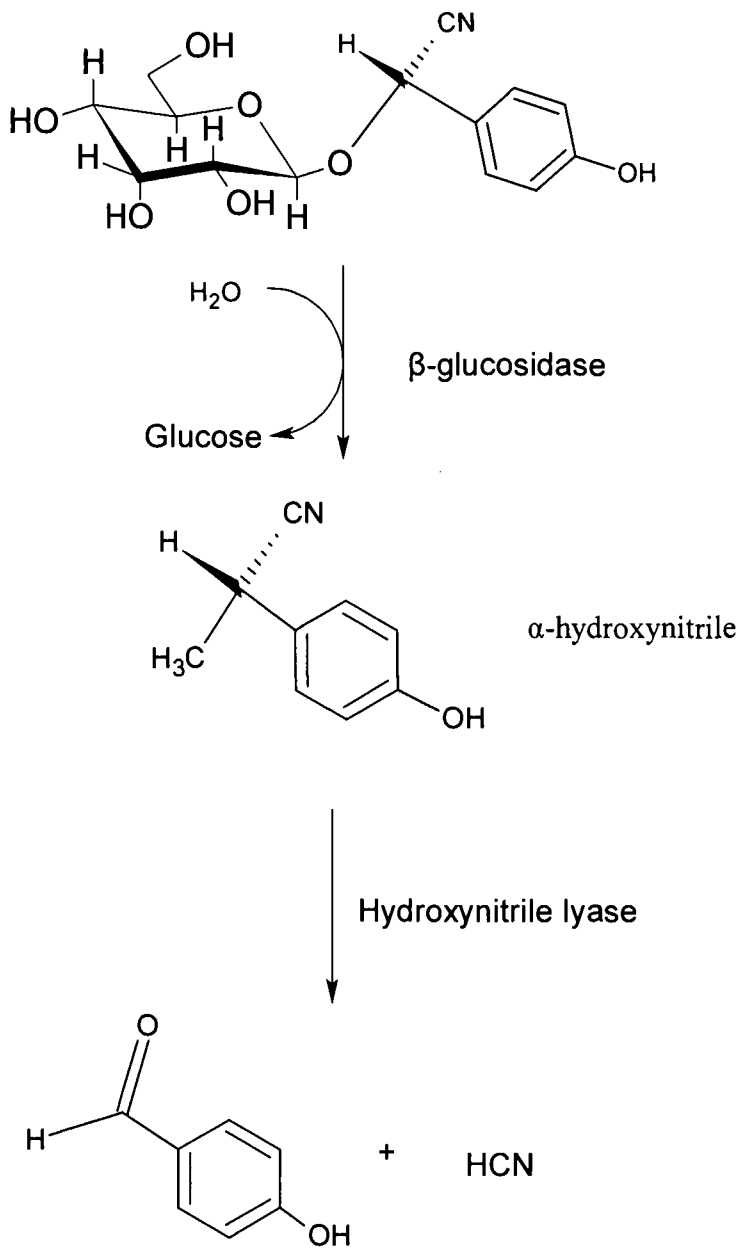
The use of FRAP for determining the antioxidant activity in fruits and vegetables has been demonstrated (*36, 68, 75, 81*). The FRAP values for fruits in the *Prunus* genus, for instance, plums, peaches, and nectarines have been studied by Gil et al. (*36*) and Proteggente et al. (*81*). Red plums (20.6 μmoles TE/g) has higher FRAP value than apples (3.9 μmoles TE/g), peaches (3.4 μmoles TE/g), and pears (3.2 μmoles TE/g) but lower than strawberries (33.5 μmoles TE/g) and raspberries (23.3 μmoles TE/g) (*81*). Gil et al. (*36*) evaluated FRAP of peaches,

nectarines and plums expressed as 13 to 107.3, 7.1 to 88.7, and 27.4 to 61.1 mg ascorbic acid equivalent/100g, respectively. No information on antioxidant activity of cherries as measured by the FRAP assay has been reported.

TOXIC SUBSTANCE IN CHERRIES

Cyanogenic glycosides are plant secondary metabolites that are found in several fruits and vegetables including cherries and apricots (*90, 96*). The toxicity of this compound has been attributed to their degradation product, hydrogen cyanide (*90, 96*). Cyanogenic glycosides consist of an α-hydroxynitrile, stabilized by glucose. Once plant tissues are damaged, the cyanogenic glycosides and their hydrolytic enzyme were leached out and hydrolysis occurs. The hydrolysis of cyanogenic glycosides involves two steps (*90*) (Figure 1.9). First, cyanogenic glycosides are cleaved by β-glucosidases. Then, the hydroxynitrile lyases catalyze the decomposition of the resulting cyanohydrins to carbonyl compounds and free hydrogen cyanide. The toxicity of hydrogen cyanide and cyanide can result in an inhibition of respiration (*90*). The lethal does of cyanide for human is considered as 1 mg per kg body weight (*90*). Cherry juice provided approximately 0.5-1.9 mg/kg of cyanide after complete cyanogenic glucoside hydrolysis (*90*). Cyanogenic glycosides are located in cherry pits (*2*).

Figure 1.9: Hydrolysis of cyanogenic glycosides in plants



CHANGES OF ANTHOCYANINS AND POLYPHENOLICS DURING PROCESSING AND STORAGE

Cherries, especially sweet cherries, are primarily grown for fresh consumption. However, processing plays an important role for cherry availability during the off-season such as frozen storage, canning, brining, and dehydrating (*46*).

Frozen storage

Frozen sweet cherries are a popular ingredient for yogurt and dairy-based dessert items. However, one study has investigated the effect of frozen storage on sweet cherry anthocyanins and color stability (*79*). Polesello and Bonzini (*79*) investigated the anthocyanin composition and color stability in dark red sweet cherries during four-month storage at –20 °C. During frozen storage, the losses of anthocyanins (34-73%) as well as the presence of anthocyanidins were observed. The formation of anthocyanidins can be attributed to the anthocyanin hydrolysis during storage. Cherry color shifts from red to purple-red and the decrease in lightness suggest the beginning of browning. However, since there is no pronounced effect in the cherry visual appearance, the loss of anthocyanins was suggested to occur in the pulp rather than in the skins, either from drip loss or anthocyanin degradation.

Pifferi and Cultrera (*78*) isolated the skin and flesh polyphenoloxidase from frozen sweet cherries stored at –20 °C. The anthocyanin destructive effect of

polyphenoloxidase depends on the presence of phenols, for instance, chlorogenic acid, catechin, and pyrocatechol. Manzocco et al (*60*) investigated the rate of polyphenoloxidase and peroxidase reaction in sucrose-, fructose- and glycerol-water media in the temperature ranged 20 to -30°C. Lower temperature decreases the rate of enzyme activity.

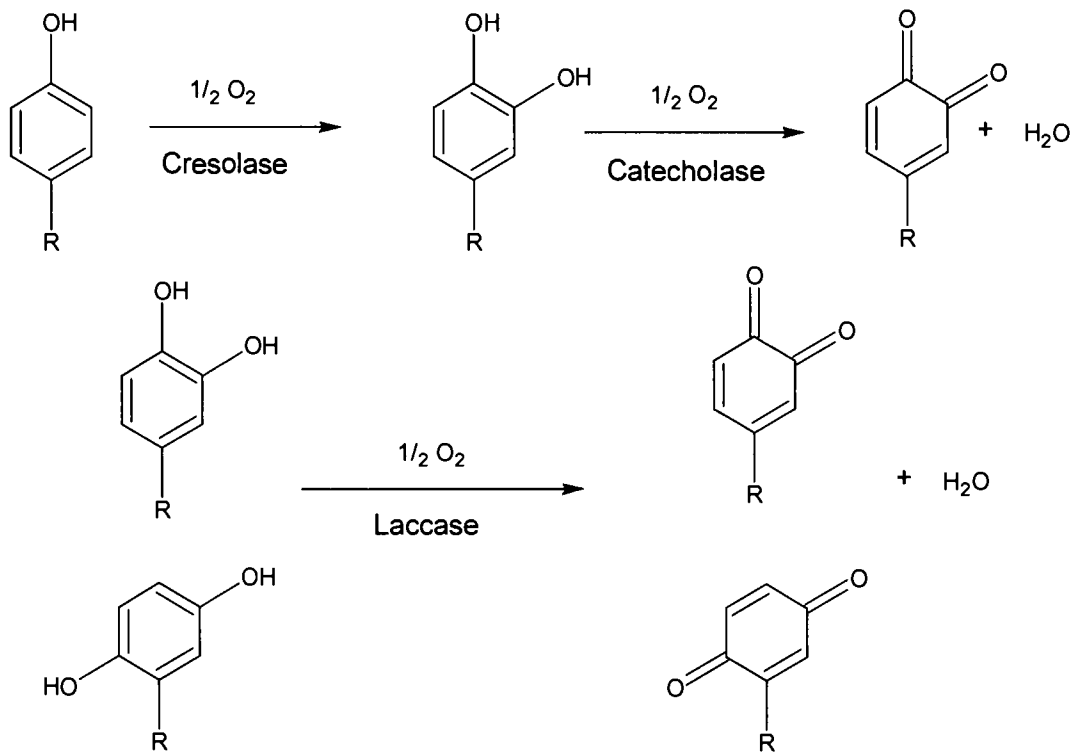
Enzymatic browning plays a significant role of anthocyanin and polyphenolic degradation in cherries and other fruits and vegetables primarily because of polyphenoloxidase activity (*47, 78, 110*). Polyphenoloxidase is the group of enzymes catalyzing polyphenolic oxidation to colored quinone formation in the presence of oxygen (*84*). Quinones are highly reactive compounds and result in the degradation of anthocyanins (*30, 47*). Furthermore, the quinones undergo oxidative polymerization to form melanoidins, brown-colored pigments. The melanin-related products from enzymatic browning possess antioxidant properties such as hydrogen donor, free radical scavenger, and lipoxygenase inhibition (*24*). The sweet cherry polyphenoloxidase were studied by Pifferi and Cultrera (*78*) and Benjamin and Montgomery (*11*).

Nicolas et al. (*73*) described the reaction catalyzed by polyphenoloxidase (Figure 1.10). In the first reaction catalyzed by mono-phenol mono-oxygenase (EC 1.14.18.1) also known as tyrosinase, phenolase, and cresolase, monophenols are hydroxylated to *o*-diphenols. Then, the oxidation of *o*-diphenols into *o*-quinones is catalyzed by catechol oxidase (EC 1.10.3.1) also known as diphenol oxidase, *o*-diphenolase, phenolase, polyphenoloxidase, and tyrosinase. Laccases (EC

1.10.3.2) oxidize *o*-diphenols as well as *p*-diphenols to quinones. The laccases are reported in peaches and apricots (*30*).

Kader et al. (*47*) proposed a mechanism for the degradation of cyanidin-3-glucoside by blueberry polyphenoloxidase. They suggested that the degradation of cyanidin-3-glucoside is pronounced in the presence of chlorogenic acid. Chlorogenic acid quinones are formed enzymatically by polyphenoloxidase. In the presence of cyanidin-3-glycoside, cyanidin-3-glycoside is degraded by a coupled oxidation mechanism with partial regeneration of chlorogenic acid. The ratio of degraded cyanidin-3-glucoside to oxidized chlorogenic acid is equal to two.

Figure 1.10: The reaction catalyzed by polyphenoloxidase (Adapted from Robards et al.(*84*)



Canning

In 1999-2001, canning accounted for 12% of processed cherries in the USA (*1*). Sweet cherries are primarily canned with syrup while sour cherries are canned for used as prepared pie filling (*46*). Changes in cherry anthocyanins, polyphenolics and antioxidant activity during canning and storage have not been investigated. A few studies has been conducted in other *Prunus* spp., for example, plums (*108*) and peaches (*7*).

Enzymatic browning does occur during canning preparation. Once cherries are bruised or pitted, the polyphenolics and several native enzymes, especially polyphenoloxidase, are released. Polyphenoloxidase plays a major role in the polyphenolic degradation and the brown pigment formation (*11, 48, 78, 84*). Eliminating oxygen or adding reducing agents such as sodium bisulfite and ascorbic acid retards the action of polyphenoloxidase.

The effect of canning on plum anthocyanins was investigated by Weinert et al. (*108*). During heating plum cell membrane and cell structure are disturbed, resulting in a mix of cell components and a loss of turgidity. Anthocyanins from skins diffuse to syrup. The loss of anthocyanins is found during pasteurization (4%) and during 47 day storage at 4 °C (13.5%) and 30 °C (46%) (*108*). To explain the loss of plum anthocyanins, Weinert et al (*109*) studied the polymer concentration in plum skin and canned liquid during plum canning. They reported that the monomeric anthocyanins decrease by 30% resulting in the increase of di and polymer forms. However, changes in polymer concentration account for a

small part of pigment loss. Other factors including the full degradation or irreversible binding of anthocyanins should be considered (*109*).

Markakis (*61*) summarized the thermal degradation of anthocyanins in 2 pathway. In the first pathway, heating shifts the equilibrium of quinonoid or flavylium through colorless carbinol psuedobase to colorless chalcone and coumarin glycoside. The second pathway involves the hydrolysis of glycosidic bond to form anthocyanidins. Then, aglycone converts to a highly unstable α-diketone intermediate and then to aldehydes and benzoic acid derivatives. These compounds can lead to the formation of brown-colored pigments (*76*).

Tanchev and Joncheva (*93*) reported that the thermal degradation of cyanidin-3-rutinoside and peonidin-3-rutinoside in cherries proceeds as a first order reaction. Partial hydrolysis of cyanidin-3-rutinoside to cyanidin-3-glucoside was observed during pasteurization of osmodehydrated cherries (*31*). In addition, the degradation products of cyanidin glycosides such as protocatechuic acid, 2, 4-dihydroxybenzoic acid, and 2, 4, 6-trihydroxybenzoic acid from tart cherries have been detected after microwave heating or boiling to 100 °C for 1 hour (*87*).

Changes of peach polyphenolics during canning were studied by Asami et al. (*7*). Several combinations of processing time and temperatures had also been investigated. Thermal processing at 104 °C for 10 min resulted in the reduction in total phenolics (21%), procyanidins monomers (49%), procyanidin dimer (88%), and procyanidin oilgomers (100%). The low temperature long time (100 °C for 40

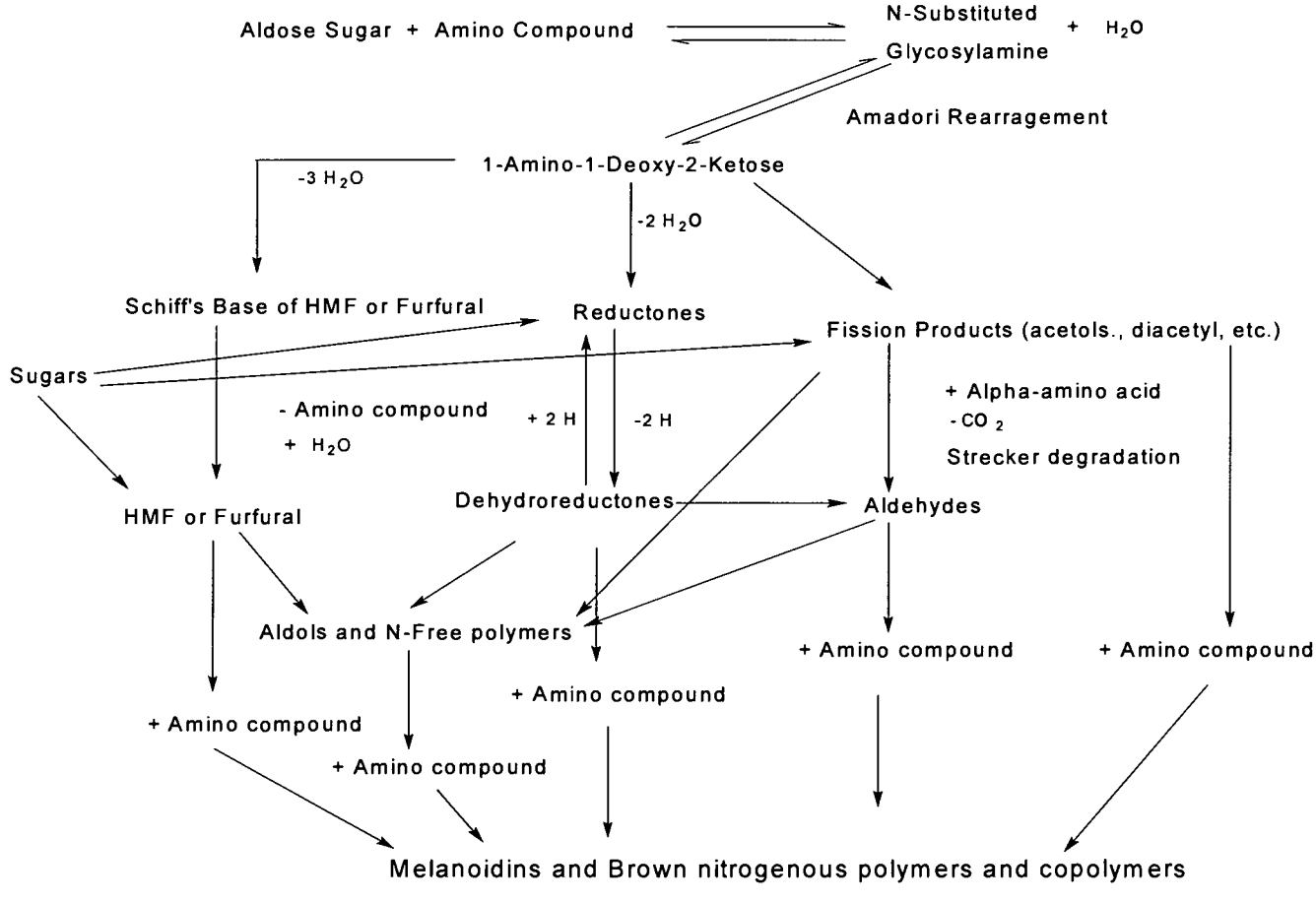
min) prevents the loss of total phenolics. A 30-43% loss in total phenolics is found during the first 3 month of storage at room temperature.

Heating causes an increase in the overall antioxidant potential of tomato juice as the consequence of the formation of melanoidins during the advanced step of Maillard reaction (*4*). The Maillard reaction consists of several interconnected processes (Figure 1.11) (*41, 74*). The first step of Maillard browning involves condensation between the carbonyl groups of reducing sugars and the α-amino group of amino acids, peptides, and proteins (*30, 74*). An initial product is a compound which immediately loses water to form a Schiff base followed by cyclization to form N-substituted glycosylamine (*30, 74*). The N-substituted glycosylamine is extremely unstable and undergoes the Amadori rearrangement or the Heyns rearrangement, yielding Amadori compounds and Heyns compounds (*30, 74*). The subsequent breakdown of Amadori compounds and presumably also Heyns compounds form the reductone-like compounds and melanoidins (Figure 1.11) (*29, 30, 42*). The presence of reductones were reportedly associated with antioxidant activity since enediol structure can be easily oxidized (*29, 56*). The antioxidant property of Maillard reaction products is related to the chain breaking and oxygen scavenging activities (*4*).

The antioxidant properties of Maillard reaction intermediate and Maillard reaction products have been studied and reviewed (*4, 29, 56, 62*). The increase of antioxidant property in tomato juice after heating coincided with the formation of brown Maillard reaction products (*4, 29*). Mastrocola and Munari

(*62*) demonstrated that antioxidant activity has been developed with increased browning in preheated model systems during storage. The application of Maillard reaction products as antioxidants in foods such as cookies and frozen sausages was reviewed in Lingnert and Eriksson (*56*).

Figure 1.11: The Maillard reaction (Adapted from Eskin (*30*))

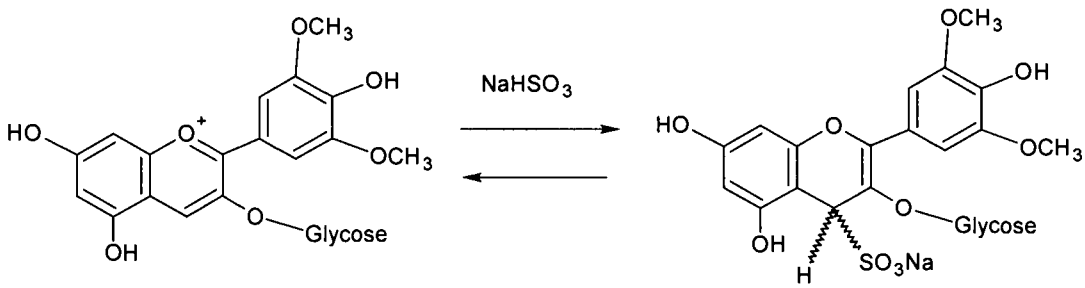


Brining

Maraschino cherries are a major cherry product which is widely distributed around the world. The maraschino cherry processing and chemical changes during processing are described in Wrolstad (*111*). In maraschino processing, cherries are brined with sulfite primary bleaching brine and chlorite secondary bleaching brine. The purpose of the sulfite primary bleaching brine is to inhibit microbial growth by sodium metabisulfite and to firm cherry texture by calcium crosslinking with pectin. The purpose of chlorite secondary bleaching brine is to bleach brown discoloration from enzymatic browning.

Not only does the sulfite function as a microbial inhibitor, it also effectively inhibits enzymatic browning. The sulfite acts as a reducing agent, which can reduce quinones, melanoidin-precusors to the colorless diphenols, and prevent the polyphenolic oxidation (*63*). The effect of bisulfite on anthocyanins is to form colorless compounds (*15*) (Figure 1.12). The decolorization is reversible. Heating and acidifying to a low pH regenerates anthocyanins by releasing sulfur dioxide (*45*). The effect of brining on anthocyanin and polyphenolic composition and antioxidant activity has not been previously investigated.

Figure 1.12: The reaction of anthocyanins with sodium metabisulfite to form the colorless compounds (Adapted from Berke et al.(*15*))



Dehydrating

Dehydrated sweet and sour cherries are considered specialty products in North America since a limited quantity is produced (*46*). Several researchers investigated the effect of drying on cherries (*28, 31*) and plums, fruits in *Prunus* spp. (*82, 83*).

Do et al. (*28*) evaluated the effect of freeze-drying on anthocyanins in Montmorency. Thirty percent of anthocyanins were lost during freeze-drying. After 6 month storage, a 62 % and 92% loss in anthocyanins were found in freeze-dried cherries stored at 21 °C and 38 °C, respectively. The higher the storage temperature is, the more anthocyanin degradation is pronounced. Cyanidin-3-(2^{G}-xylosylrutinoside) was the least stable. The use of osmotic dehydration of sweet cherries along with pasteurization was investigated (*31*). After 2 hours of osmotic

dehydration, 6% of cherry anthocyanins were lost resulting in slight lightening of cherry color.

Raynal and Moutounet (*82*) and Raynal et al. (*83*) investigated the effect of conventional air drying on plum anthocyanins and polyphenolics. In high drying temperatures such as 95 °C, flavonoids such as rutin and anthocyanins are degraded while the hydroxycinnamic acids are preserved since polyphenoloxidase is denatured (*83*). The mechanism of anthocyanin degradation was related more to quinone formation in the presence of chlorogenic acid and polyphenoloxidase than to thermal degradation.

CONCLUSIONS

Sweet and sour cherries are widely grown in the USA. Sweet cherries are primarily produced in the West Coast States: Washington, Oregon, and California while sour cherries are mainly grown in Great Lakes States, mainly in Michigan. Epidemiological evidence shows that increased consumption of fruits and vegetables can reduce the risk of several chronic diseases such as heart disease, cancer, and stroke due to natural antioxidants; for instance, polyphenolics.

A number of studies and reviews on anthocyanin and polyphenolic composition as well as changes of anthocyanins and polyphenolic during processing and storage have been summarized in this chapter. The variations are recognized due to the genetic, environmental, post harvest, and processing factors as well as methodology in extraction and analyses.

Antioxidant activities of sweet and sour cherries have also been reviewed. Several methods have been used to evaluate the antioxidant activity in sweet and sour cherries, making comparison difficult. In addition, the antioxidant activities of sweet cherries, especially light-colored sweet cherries: Royal Ann and Rainier, and the effect of processing and storage on antioxidant activity have not been thoroughly investigated.

The comprehensive investigation on sweet and sour cherries and the relationship between polyphenolics and antioxidant properties have been conducted in this study. The distribution of anthocyanin and polyphenolic content and composition along with antioxidant activities in skins, flesh, and pits of 3 sweet cherry cultivars and 1 sour cherry cultivar were studied. Changes of sweet cherry anthocyanins, polyphenolics, and antioxidant activity during processing and storage were monitored.

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CHAPTER III

ANTHOCYANIN PIGMENTS AND TOTAL PHENOLIC CONTENT OF FRESH AND PROCESSED CHERRIES AND THEIR ANTIOXIDANT PROPERTIES

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ABSTRACT

Total anthocyanin pigments, total phenolics, and the antioxidant properties of three sweet cherry (*Prunus avium* L.) and one sour cherry (*P. cerasus* L.) cultivars were determined. Bing cherries were highest in anthocyanins (60.6 mg/100g fw) while Montmorency cherries were highest in both total phenolics (5.6 mg GAE/g fw) and antioxidant activities [Oxygen Radical Absorbance Capacity (ORAC) 51.02 μmoles Trolox equivalent (TE) /g fw, Ferric Reducing Antioxidant Power (FRAP) 47.96 μmoles TE/g fw]. Total phenolics and anthocyanins were concentrated in the skin. Over 75% of the anthocyanins in frozen Bing cherries were destroyed after 6 months storage at –23 °C while they were reasonable stable at –70 °C. During canning, about half of the anthocyanins and polyphenolics leached from the fruits into the syrup with little total loss. Spent cherry brine contained substantial anthocyanins and polyphenolics. Negligible amounts remained in the fruits after the brining and washing operations. Frozen storage and canned storage had less impact on total phenolics than on anthocyanins.

Keywords: cherries, anthocyanins, total phenolics, canning, brining, frozen storage, antioxidant properties, ORAC, and FRAP

INTRODUCTION

Epidemiological studies show a strong association between fruit and vegetable consumption and reduced the risk of several degenerative diseases such as cancer, cardiovascular disease, and stroke that are caused by oxidative stress (*2, 25*). Specifically, cherry consumption has been reported to alleviate arthritis and gout-related pain (*35, 44*). The beneficial effects from fruits and vegetables have been ascribed to natural antioxidants such as anthocyanins and polyphenolics (*23, 34*). Sweet cherries (*Prunus avium* L.) and sour cherries (*Prunus cerasus* L.) contain substantial amounts of anthocyanins and polyphenolics (*12, 13, 14, 42, 43*). Comparative data on sweet and sour cherry composition using the same analytical methodologies, however, is limited.

Anthocyanins and polyphenolics are not uniformly distributed in fruit tissue. Skin contains high amounts of polyphenolics and anthocyanins (*40*). This is most likely because of their functions as photoprotective agents and attractants for seed dispersal. Anthocyanins may be distributed throughout the fruit, as is the case for raspberries, strawberries, and blackberries, or limited to the skins, as is the case for apples and most blueberry cultivars. Anthocyanins in Bing cherries are present in skins and flesh while in Rainier, Royal Ann, and Montmorency cherries, they are limited to the skins. Royal Ann and Rainier cherries are yellow with a red blush while Montmorency cherries are intense red in skin color. The flesh of all three is yellow colored. Quantitative information on the distribution of cherry anthocyanins, total phenolics, and their antioxidant properties is not available.

Cherry skins with their high anthocyanin and polyphenolic content could be a potential source for nutraceuticals and natural antioxidants, as so could juice processing wastes, which include skin as well as pits.

Several methods have been used to determine the antioxidant activity of fruits (*16, 19, 27, 35*). Heinonen et al. (*16*) compared the *in vitro* antioxidant activity of several fruits. Sweet cherries have higher antioxidant activity than blueberries and strawberries and are ranked as the first and the third in their antioxidant activities in liposome and low-density lipoprotein, respectively. Seeram et al. (*35*) reported that the anti-inflammatory activity of sweet cherries was higher than Montmorency cherries.

Oxygen Radical Absorbance Capacity (ORAC) and Ferric Reducing Antioxidant Power (FRAP) have been used to determine "total antioxidant activity" for several fruits including blueberries, raspberries, black currants, blackberries, apples, and pears (*20, 21, 22, 29, 41*). The two methods differ with respect to their chemical basis. FRAP measures the ability of an extract or compound to reduce the ferric ion while ORAC measures the ability to scavenge free radicals (*31*).

In the USA, the cherry season is short, lasting from Mid-May to Mid August across the entire country, and for a few weeks for any specific region. In 1999-2001, about 40% of sweet cherry production were brined (70%), canned (12%), and frozen, dried, or used for juice (18%) (*1*). Approximately 99% of tart cherry crop were frozen (> 50%), canned (>33%), and brined, dried, or used for juice (>10%) (*1*). The primary use of processed sour cherries is in baking and

cooking. While there have been several investigations on the effect of processing on anthocyanins and polyphenolics in fruits other than cherries (*21, 37*), there are no thorough studies on the impact of processing on cherry anthocyanins and polyphenolics. Most cherry processing studies are directed to anthocyanins and color quality (*9, 11, 33*). Because of their possible health benefits, there is intense interest in the impact of processing on the antioxidant properties of these compounds.

Our objectives were to determine the total anthocyanins, total phenolics, and antioxidant properties of selected cherry cultivars (Bing, Royal Ann, Rainier, and Montmorency) and measure their distribution in fruits. Evaluation of the effect of processing and storage was an additional component of this investigation.

MATERIALS AND METHODS

Sources of cherry samples

Bing, Royal Ann and Montmorency cherries were harvested at the OSU Lewis Brown horticultural farm, Corvallis, OR, from late-June to mid-July, 2001. Fresh Rainier cherries were obtained from the Mid-Columbia Experiment Station, Hood River, OR, mid- July, 2001. Oregon Cherry Growers, Inc., Salem, OR provided Bing and Royal Ann cherries for brining processing experiment in June,

2000, and also provided Bing cherries for canning and frozen storage experiments in June, 2001. Upon receipt at the Department of Food Science and Technology, Oregon State University, Corvallis, OR, the cherries were stored at 2 °C prior to sample preparation or processing.

Fresh cherries

Cherry samples (ca. 250 g) were carefully separated into skins, flesh, and pits using a stainless steel knife, weighed, and frozen in liquid nitrogen for the distribution study. Cherry samples (ca. 200 g) were also pitted using a household hand cherry pitter for studies on edible portions of cherries. Samples were prepared by two replications and stored at –70 °C until analysis.

Extraction of anthocyanins and polyphenolics

Samples were cryogenically milled with liquid nitrogen using a stainless steel Waring Blender. The powder (ca. 10 g) was mixed with 20 mL of acetone, sonicated with an ultrasonic cleaner (Branson Cleaning Equipment Corp., Shelton, CT.) for 10 min and then filtered using Whatman No.1 paper on a Büchner funnel. The filter cake was re-extracted with 10 mL of 70 % acetone (30% water and 70% acetone, v/v) twice. Filtrates were combined and mixed with 80 mL of chloroform and then centrifuged at 170 *Xg* for 20 min on a IEC International Centrifuge (Model UV, International equipment Co., Boston, MA). The upper aqueous phase was collected and evaporated *in vacuo* at 40 °C until the residual acetone residual

was removed (ca. 15 min). The aqueous extract was made up to 25 mL with acidified water and stored at –70 ° C until subsequent analyses. Sample extractions were replicated twice.

Cherry processing

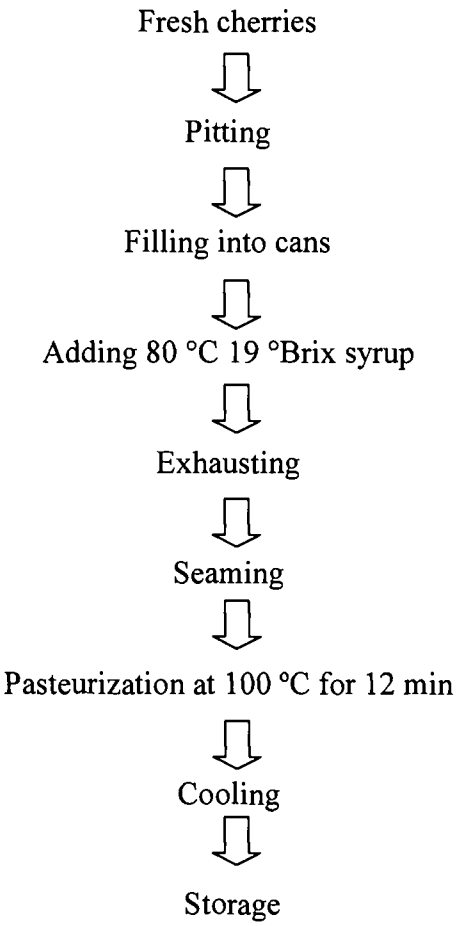
Frozen cherries

Stemmed Bing cherries (2 Kg) were washed, pitted with a household hand cherry pitter, and frozen in liquid nitrogen. The 120 g samples were packaged in 12 plastic Nalgene containers and closed with caps. Six containers were stored at -23 °C and the others at –70 °C. Analyses were conducted after 3 and 6 month storage.

Canned cherries (Figure 2.1)

Stemmed Bing cherries (2.5 Kg) were washed and pitted using a household hand cherry pitter. No.303 cans with dark fruit enamel were filled with pitted cherries and 19 °Brix sucrose syrup (C&H Superfine sugar diluted to19 °Brix with water; C&H Sugar Company, Inc., Crockett CA) at 80 °C was added. The cans were exhausted on a steam bath for 2 min 30 second and sealed with No.303 lids on a Canco 006 Steam-flow Can seamer. The cans were immersed in water and heated at 100 °C for 12 min. The canned fruit was cooled by placing in 25 °C water and then stored at 2 and 22 °C. Canning experiments were replicated twice.

Figure 2.1: The flow chart of cherry canning



Brined cherries

Royal Ann and Bing cherries with stems (about 1 Kg) were each placed in 4 L glass jars. Bisulfite brine solution was prepared by dissolving 220 g of sodium metabisulfite, 202 g of calcium chloride, and 10 g citric acid in 10 L of water. This gave a solution, which was 2.2% sodium metabisulfite, 2% calcium chloride, and 1% citric acid. The pH was about 3.0. The brine was added to the fruit-containing jars until the fruits were covered. Plastic wrap covered the glass jars which allowed for air diffusion. The brined fruits were stored for 12 months at 22 °C. The spent brine was recovered for analysis, and the cherries were washed with running cold water for about 5 days until SO_2 levels were less than 200 ppm. Washed brined cherries and spent brine were frozen with liquid nitrogen and stored at –70 °C.

Determination of free SO_2 level

The free sulfur dioxide level was measured by iodine titration as described by Beavers et al. (*5*), except that 0.02 N iodine solution was used instead of 0.156 N. Free SO_2 levels were determined for the brining solution and brined cherries after washing.

°Brix and pH

°Brix was measured using an Auto Abbe refractometer 10500 (Reichert-Jung, Leica Inc., NY). The instrument was set to measure % total soluble solids

with the temperature compensated mode. A Brinkmann 605 pH meter (Methrohm, Swtizerland) was used for pH determination.

Anthocyanin pigment content and polymeric color indices

The monomeric anthocyanin pigment content was determined using the pH-differential method (*15*). A Shimadzu 300UV spectrophotometer and 1 cm pathlength disposable cell were used for spectral measurements at 510 and 700 nm. Pigment content was calculated as mg cyanidin-3-glucoside/100g fresh weight (fw) using an extinction coefficient of 26,900 $Lcm^{-1}mol^{-1}$and molecular weight of 449.2 $gmol^{-1}$

Color density, polymeric color, and % polymeric color were determined using the bisulfite bleaching method as described in Giusti and Wrolstad (*15*).

Total phenolics

Total phenolic content was determined using the modified Folin-Ciocalteu procedure described by Singleton and Rossi (*36*). A 0.5 mL sample or a series of gallic acid standards (0, 40, 80, 120, 160, 200 ppm) were mixed with 0.5 mL of the Folin-Ciocalteu reagent (Sigma Chemical Co., St. Louis, MO) and 7.5 mL of deionized water. The mixture was held at room temperature for 10 min before adding 1.5 mL of 20% sodium carbonate (w/v). The mixtures were heated in a

Statistical analysis

The distribution of anthocyanins, total phenolics, and antioxidant activities in four cherry cultivars and the effect of processing on cherry anthocyanins, total phenolics, and antioxidant activities were analyzed by analysis of variance using S-Plus 4.5 (MathSoft, Seattle, WA). To assess the effect of frozen storage on total anthocyanins, total phenolics, and antioxidant activities, a one factor ANOVA model was used, the factor being the combination of freezing temperatures and storage time. The effect of canning and storage on anthocyanins, total phenolics, and antioxidant activities were analyzed separately. To evaluate the effect of canning on anthocyanins, total phenolics, and antioxidant activities, a one factor ANOVA model was used with the levels of the factors as follows: fresh cherries, canned cherries at two storage temperatures. To evaluate the effect of storage after canning, a two way ANOVA was used with the time and temperature levels as factors. To assess the effect of brining, a two-way ANOVA model was used with the cherry cultivars and processing as factors. The Pairwise comparisons between treatment level means in all ANOVA models were carried out using Tukey's method ($p \leq 0.05$). The Pearson correlation matrix was used to determine the correlation among anthocyanins, total phenolics, and antioxidant activities. A ninety-five percent significant level was used throughout the analyses.

RESULTS AND DISCUSSION

Distribution of cherry components by weight

The pH and °Brix of the 4 cherry cultivars are shown in Table 2.1. The proportions of skins, flesh, and pits and the physical properties of four cherries are presented in Table 2.2 and Figures 2.2-2.5. The proportions of the four varieties were similar with flesh ranging from 60.4-71.4%, skins from 13.5-18.1%, and pits from 5.5-7.9%. Inherent losses (9.6-14.7%) occurred in peeling, pitting, and weighing the samples.

Table 2.1: °Brix and pH of four cherry cultivars

Cultivars	pH	°Brix
Bing	3.94 ± 0.04	18.38 ± 0.18
Royal Ann	3.69 ± 0.02	15.49 ± 0.13
Rainier	4.11 ± 0.02	17.55 ± 0.31
Montmorency	3.52 ± 0.02	14.43 ± 0.09

Values are mean ± standard deviations, n=3

Figure 2.2: The % distribution of Bing cherries (n=3)

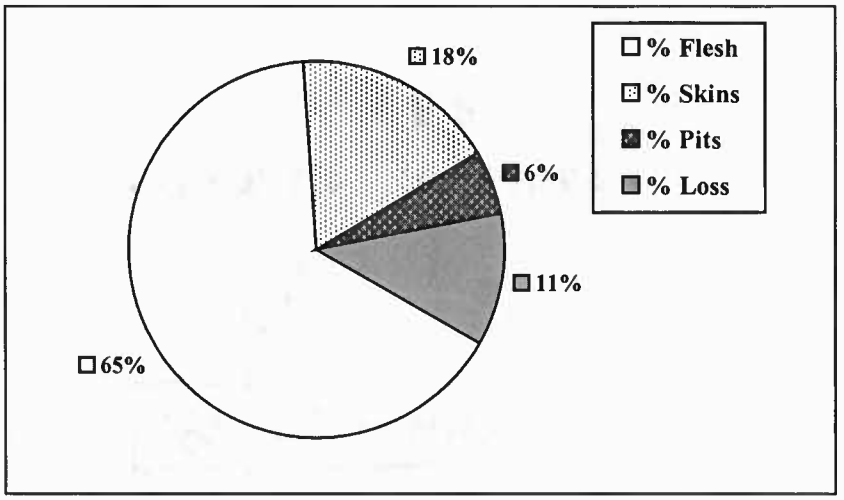


Figure 2.3: The % distribution of Royal Ann cherries (n=3)

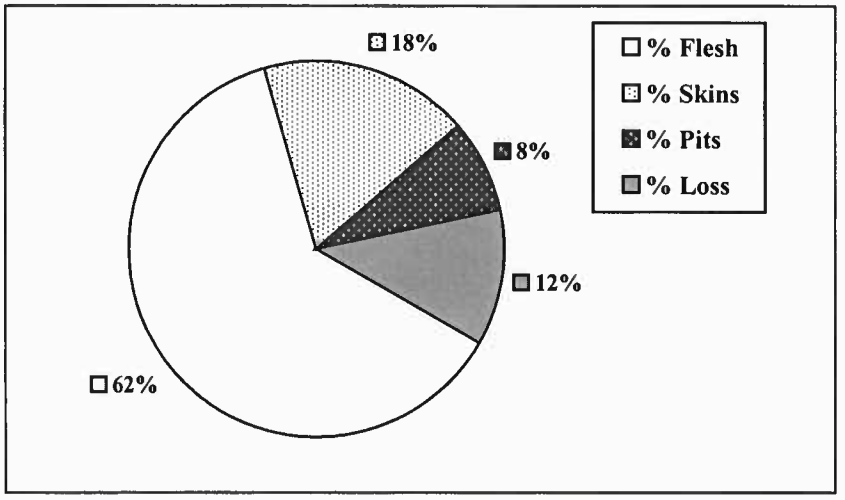


Figure 2.4: The % distribution of Rainier cherries (n=3)

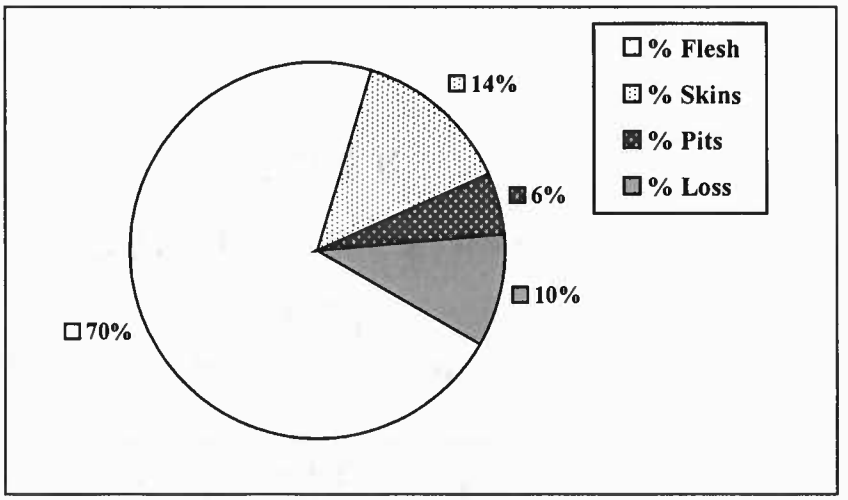
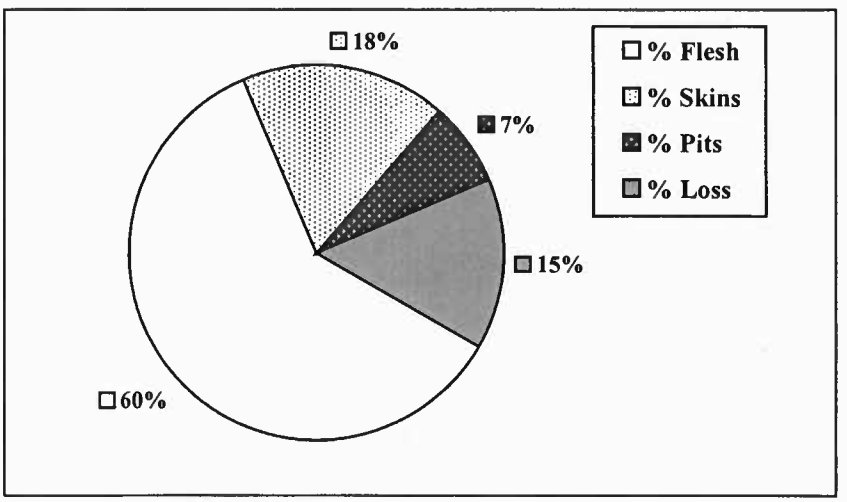


Figure 2.5: The % distribution of Montmorency cherries (n=3)



Distribution of anthocyanin pigments

Table 2.2 and Figure 2.6 present the anthocyanin content of the four cherry cultivars and their distribution in skins, flesh and pits for each cultivar. Bing was the only cultivar that had pigmentation in skins, flesh and pits, while others had pigmentation in skins and pits. The anthocyanins in pits were due to the contamination from Bing skins and flesh as well as skins from the others. The highest anthocyanin concentration (60.6 mg/100g) was found in Bing skins. The brilliant red skin of Montmorency cherries had the second highest anthocyanin concentration (36.5 mg/100g). Royal Ann and Rainier cherries are yellow colored with a red blush and had light anthocyanin pigmentation, 2.2 and 2.1 mg/100g, respectively.

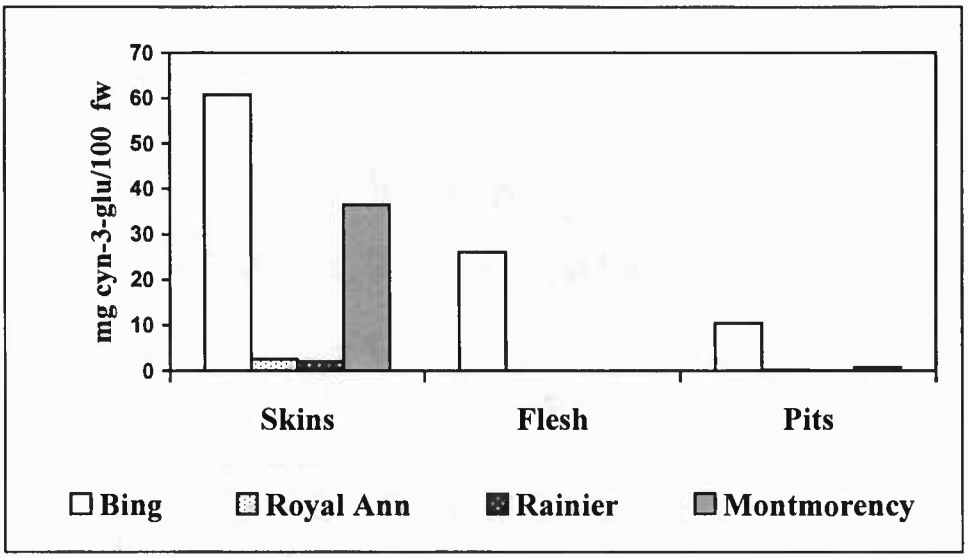
Anthocyanin content of the edible portion was highest for Bing cherries (29.7 mg/100g), followed by Montmorency (8.7 mg/100g), Rainier (0.5 mg/100g), and Royal Ann cherries (0.5 mg/100g). There can be considerable variation in pigment content from lot to lot. Other lots of Bing cherries used for our processing experiments had levels as high as 63.7 mg/100g. These values for Bing cherries are much lower than the value (224.7 mg/100g) reported by Gao and Mazza (*14*), but comparable to the quantity (25.95 mg/100g) reported by Seeram et al. (*35*). The amounts of Montmorency anthocyanins in our study were lower than the values (12.5 to 25.0 mg/100 g) reported by Wang et al. (*42*).

Table 2.2: Total anthocyanins, total phenolics, and antioxidant properties of edible portion, flesh, pits, and skins of four cherry cultivars

Cultivars	Portion	% Distribution	Anthocyanins (mg/100g fw)	Total Phenolic (mg/gfw)	ORAC (μmoles/g fw)	FRAP (μmoles/g fw)
Bing	Edible portion		29.7 ± 2.3	1.85 ± 0.13	14.94 ± 0.90	15.90 ± 1.42
	Flesh	65.5 ± 3.2	26.0 ± 0.7	1.34 ± 0.18	9.07 ± 0.35	7.28 ± 0.24
	Pits	5.7 ± 0.4	10.4 ± 3.1	0.92 ± 0.09	5.94 ± 0.91	5.04 ± 0.96
	Skins	17.5 ± 0.5	60.6 ± 2.5	3.33 ± 0.41	28.26 ± 1.10	21.05 ± 0.55
	Loss	11.3 ± 2.8				
Royal Ann	Edible portion		0.5 ± 0.2	2.29 ± 0.10	14.49 ± 2.20	15.53 ± 0.48
	Flesh	62.2 ± 0.7	0.1 ± 0.10	1.76 ± 0.03	13.10 ± 0.44	9.03 ± 0.19
	Pits	7.9 ± 0.1	0.2 ± 0.05	1.04 ± 0.08	5.68 ± 0.53	4.98 ± 0.36
	Skins	18.1 ± 0.2	2.2 ± 1.1	3.51 ± 0.13	27.44 ± 1.66	17.08 ± 1.11
	Loss	11.8 ± 0.6				
Rainier	Edible portion		0.5 ± 0.04	0.75 ± 0.02	4.98 ± 0.51	2.92 ± 0.26
	Flesh	71.4 ± 0.8	0.0 ± 0.0	0.65 ± 0.05	4.62 ± 0.18	2.27 ± 0.22
	Pits	5.5 ± 0.3	0.1 ± 0.0	0.54 ± 0.04	3.38 ± 0.26	2.00 ± 0.13
	Skins	13.5 ± 0.8	2.1 ± 0.4	1.42 ± 0.05	10.50 ± 1.51	5.92 ± 0.39
	Loss	9.6 ± 0.7				
Montmorency	Edible portion		8.7 ± 0.80	4.07 ± 0.18	25.57 ± 3.99	37.56 ± 0.95
	Flesh	60.4 ± 1.9	0.0 ± 0.09	3.01 ± 0.29	15.00 ± 1.00	13.81 ± 0.26
	Pits	7.2 ± 0.1	0.8 ± 0.08	1.57 ± 0.02	9.78 ± 0.28	8.48 ± 0.85
	Skins	17.7 ± 0.2	36.5 ± 1.6	5.58 ± 0.33	51.02 ± 1.97	47.96 ± 1.33
	Loss	14.7 ± 1.7				

Values are mean ± standard deviations, n=3 for % distribution while n=2 for anthocyanins, total phenolics, ORAC, and FRAP.

Figure 2.6: The distribution of anthocyanin content in four cherry cultivars (mg cyanidin-3-glucoside/100g, n=2)



The total anthocyanin content for both Rainier and Royal Ann cherries was lower than the amounts for "light-colored" cherries (2 to 41 mg/100g) reported by Gao and Mazza (*14*).

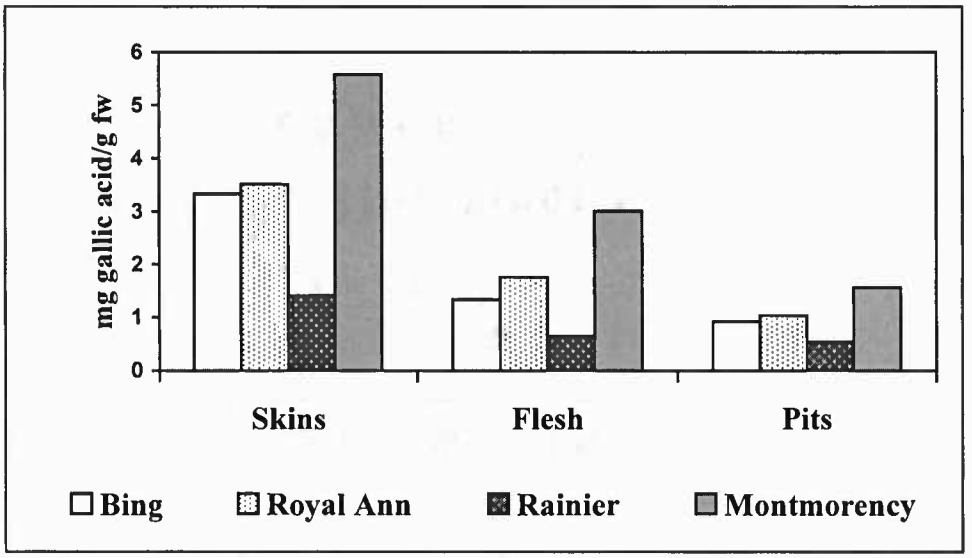
Several factors can influence anthocyanin content in cherries such as cultivar, maturity, geographic location, and environment factors such as light, temperature, and various stresses (*20, 39*).

Distribution of total phenolics

Table 2.2 and Figure 2.7 show the distribution of total phenolics in skins, flesh, and pits for the four cherry cultivars. Total phenolics were the highest in skins, intermediate in flesh, and lowest in pits. Montmorency cultivar had the highest amounts in all three portions. While Bing skins are much higher in anthocyanins than Royal Ann skins, the total phenolic levels for both skins are very similar.

Total phenolics for the edible portion of Montmorency cherries was 4.07 mg/g, followed by Royal Ann (2.29 mg/g), Bing (1.85 mg/g), and Rainier cherries (0.75mg/g). Mozetič et al. (*30*) reported that the total phenolics of Bing and Royal Ann to be 0.97 and 1.44 mg/g, respectively. Heinonen et al. (*16*) reported the total phenolics of Bing cherries as 0.80 mg/g with hydroxycinnamates being the major polyphenolic class of compounds. Our values for total phenolics are considerably higher than both of those studies.

Figure 2.7: The distribution of total phenolics in four cherry cultivars (mg gallic acid equivalent/g, n=2)



Antioxidant activities of cherry components

Cherry skins were the highest in antioxidant activities while pits were found to be the lowest (Table 2.2 and Figures 2.8 and 2.9). Results of ORAC and FRAP followed the same trends, the two measurements having a positive correlation of r = 0.98. Montmorency skins were the highest in ORAC and FRAP, following by Bing, Royal Ann, and Rainier skins, respectively (Table 2.2). Obviously, anthocyanin content *per se* is not a predictor of relative antioxidant activities as Royal Ann skins were comparable to Bing Skins in ORAC and FRAP. In addition, Royal Ann flesh, which contained essentially no anthocyanin, had higher antioxidant values than Bing flesh. Correlation between anthocyanins and ORAC

Figure 2.8: The distribution of ORAC in four cherry cultivars (μmoles Trolox equivalent/g, n=2)

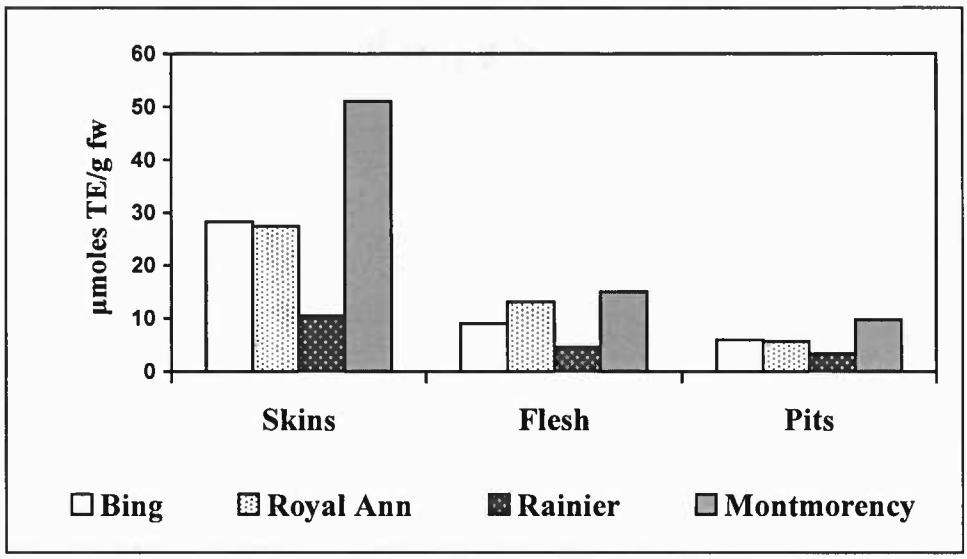
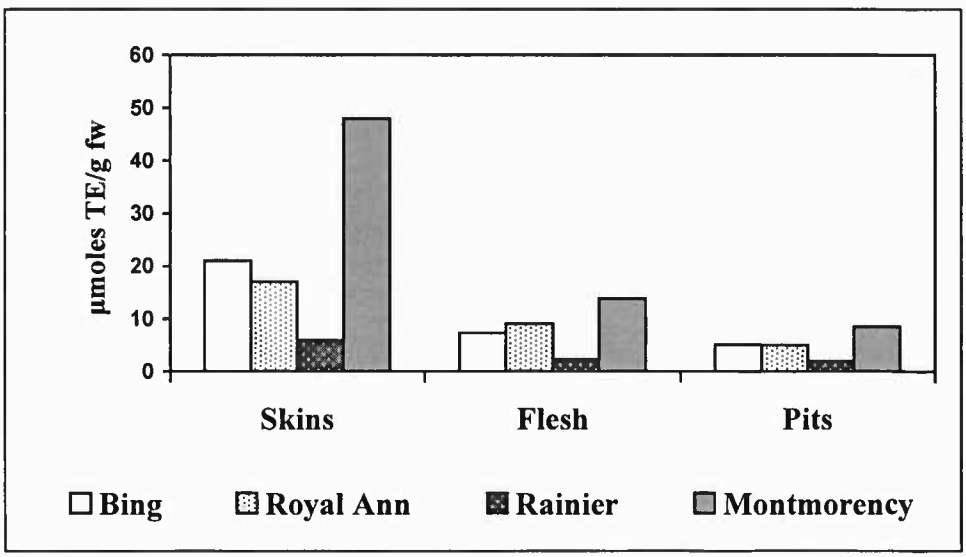


Figure 2.9: The distribution of FRAP in four cherry cultivars (μmoles Trolox equivalent/g, n=2)



and FRAP was 0.61 and 0.60, respectively. Total phenolics were a much better predictor of antioxidant properties with a positive correlation between total phenolics and ORAC of r = 0.97, and between total phenolics and FRAP of r = 0.95. Other studies have reported high correlations between antioxidant activities and total phenolics for a number of fruits (*20, 27, 29, 41*).

Effect of frozen storage

Table 2.3 and Figures 2.10-2.14 present total anthocyanins, % polymeric color, total phenolics, and the antioxidant activities of fresh-pitted Bing cherries as well as their changes after 3 and 6 months storage at –23 °C and –70 °C. Anthocyanins underwent pronounced degradation during storage at –23 °C with 66% degradation after 3 months and 87% after 6 months. Storage at –70 °C resulted in much greater anthocyanin stability with 90% remaining after 3 months and 88% after 6 months. Anthocyanin degradation is also evident from the increase in % polymeric color, from 12% in fresh cherries to 61% in cherries stored at –23 °C for 6 months.

Table 2.3: Effect of frozen storage temperature and time on total anthocyanins, % polymeric color, total phenolics, ORAC, and FRAP of pitted Bing cherries

Storage Temperature (°C)	Storage Time (month)	Anthocyanins (mg/100g fw)	% Polymeric	Total phenolics (mg/g fw)	ORAC (μmoles/g fw)	FRAP (μmoles/g fw)
Fresh	0	63.7 ± 0.9a	12.5 ± 2.3d	1.94± 0.17a	13.12 ± 1.22b	14.32 ± 0.39c
-23	3	21.5 ± 2.5c	46.3 ± 2.6b	1.45 ± 0.18b	12.57 ± 1.81bc	9.95 ± 0.69 d
	6	8.02 ± 1.0d	60.8 ± 2.8a	0.96 ± 0.04c	9.41 ± 0.95c	5.98 ± 0.21e
-70	3	57.4 ± 2.9b	17.8 ± 1.6c	2.10 ± 0.04a	24.60 ± 3.41a	16.70 ± 0.22b
	6	56.4 ± 4.0b	14.5 ± 2.0cd	2.00 ± 0.19a	23.78 ± 1.01a	20.85 ± 1.81a

Values are mean ± standard deviations, n=2. Different letters within the same column indicate significantly different at $p \leq 0.05$

Figure 2.10: Effect of frozen storage temperature and time on anthocyanin content of pitted Bing cherries (mg cyanidin-3-glucoside/100g, n=2)

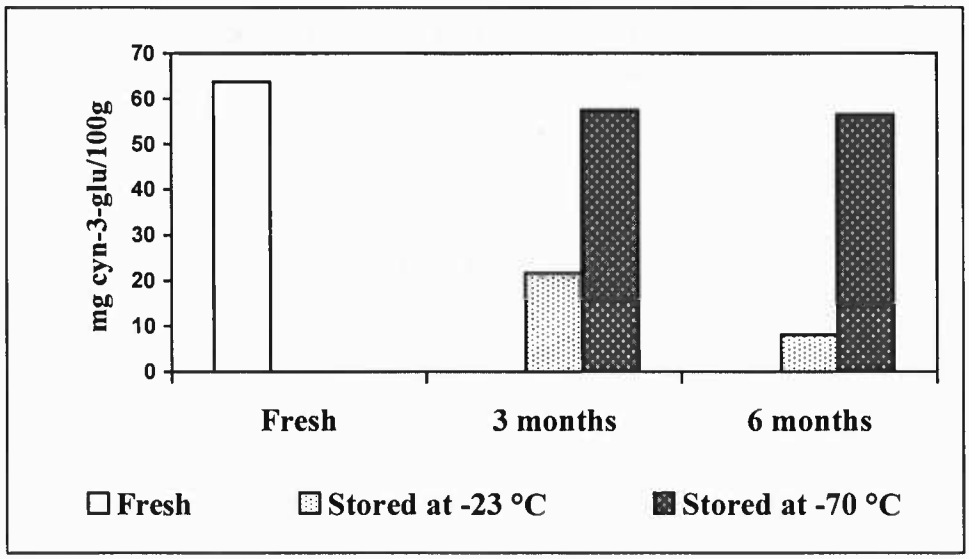


Figure 2.11: Effect of frozen storage temperature and time on % polymeric color of pitted Bing cherries (n=2)

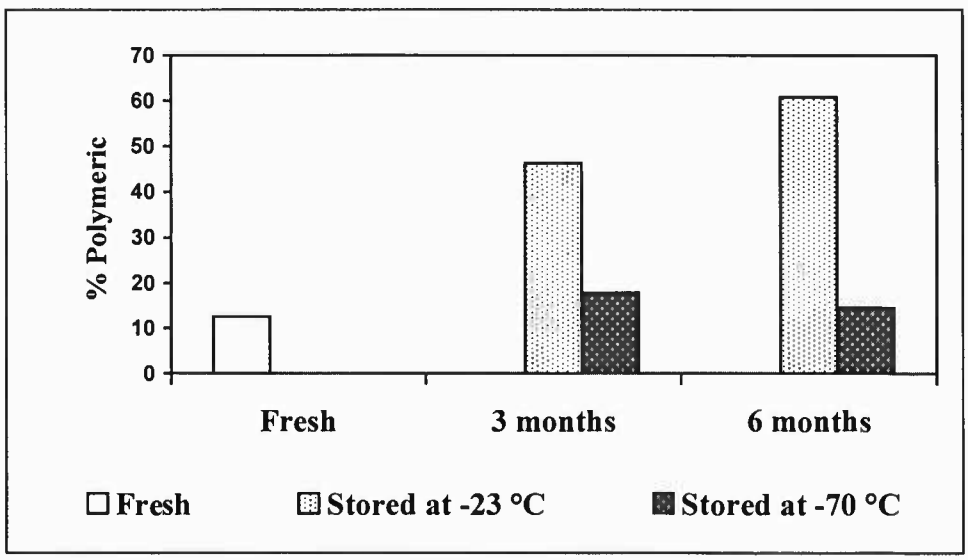


Figure 2.12: Effect of frozen storage temperature and time on total phenolics of pitted Bing cherries (mg gallic acid equivalent/g, n=2)

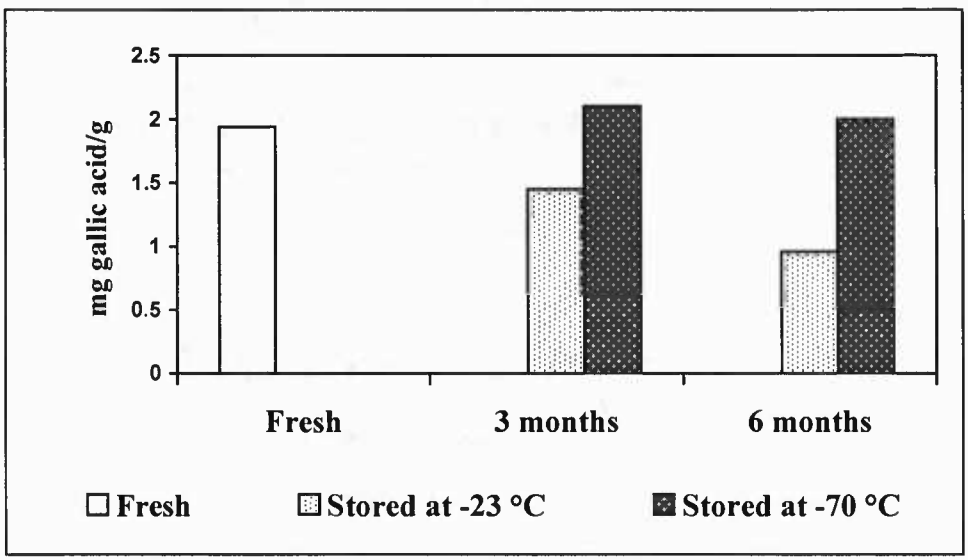


Figure 2.13: Effect of frozen storage temperature and time on ORAC of pitted Bing cherries (μmoles Trolox equivalent/g, n=2)

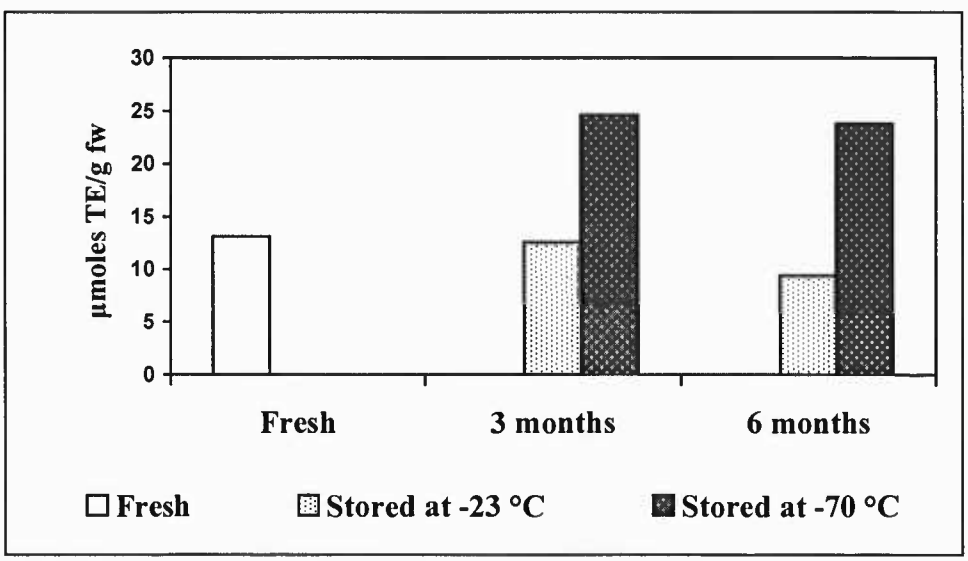
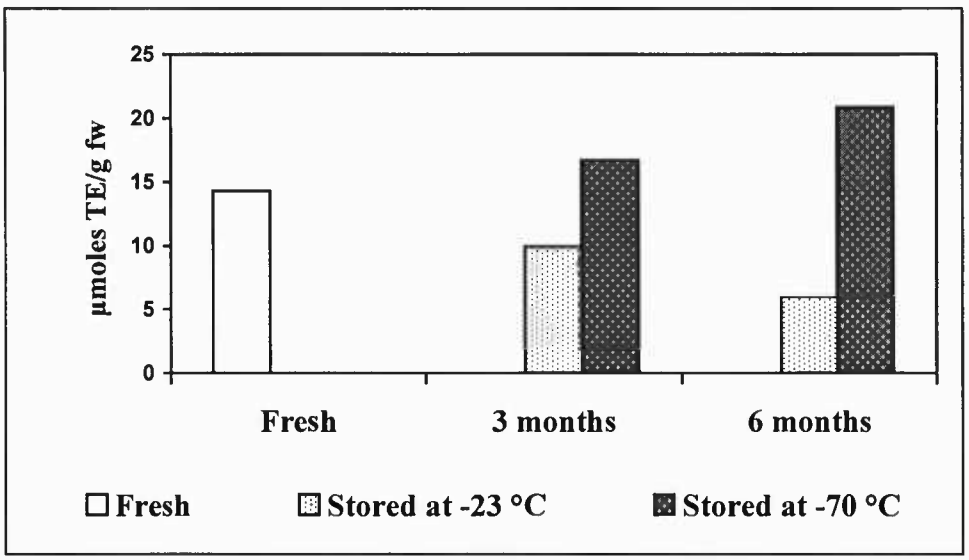


Figure 2.14: Effect of frozen storage temperature and time on FRAP of pitted Bing cherries (μmoles Trolox equivalent/g, n=2)



Anthocyanin degradation in frozen cherries is very likely related to presence of native enzymes, particularly polyphenoloxidase, which has been shown to be very active in both cherry flesh and skins (*32*). Polyphenoloxidase can accelerate anthocyanin degradation in the presence of polyphenolics, particularly chlorogenic acid which is one of the major phenolic compounds in cherries (*32*). Polyphenoloxidase activity is temperature dependent (*26*). Kader et al. (*17, 18*) showed that in blueberries, polyphenoloxidase oxidizes chlorogenic acid to a quinone which will couple with anthocyanins in a degradation reaction. Chlorogenic acid is then partially regenerated and can continue to serve as a substrate for polyphenoloxidase. This mechanism would be consistent with the

difference in the magnitude of total phenolics and anthocyanins during storage at –23 °C.

Total phenolics also decreased considerably during storage at –23 °C, about 25% degradation after 3 months, and 50% after 6 months, although not as extremely as anthocyanins. There was an apparent increase in total phenolics for cherries stored at –70 °C. Depolymeriztion of polyphenolics and/or increased extraction efficiency due to breakdown of cell-wall polysaccharides during freezing may be possible explanations.

A decrease in antioxidant activities (28% for ORAC and 58% for FRAP) during 6 month storage at –23 °C was found while there was a surprising apparent increase in antioxidant activity for cherries stored at –70 °C (81% for ORAC and 46% for FRAP). The reduction in antioxidant activities for cherries stored at –23 °C, however, was not nearly as great as the losses in total anthocyanins and total phenolics. One possible explanation is that anthocyanin and polyphenolic degradation products retain antioxidant activities.

Total phenolics also showed an apparent net increase at –70 °C but not to the same magnitude as antioxidant activity. Perhaps this apparent increase is an artifact of the antioxidant assays, still both ORAC and FRAP should marked increases.

Effect of canning

Changes of anthocyanin content, total phenolics, and antioxidant activity of pitted Bing cherries canned in light syrup during canning and storage are shown in Table 2.4 and Figures 2.15-2.18. Canning resulted in approximately 50% transfer of anthocyanins and total phenolics from the fruits into the syrup.

Heat processing did not result in a loss of total anthocyanins, total phenolics, and antioxidant activity when the values for syrup and cherries were combined. In fact, samples show an apparent slight increase in total anthocyanin content with canning. This might be due to increased extraction efficiency in the softened fruits. Higher temperature may increase membrane permeability in the macerated peel tissue facilitating phenolic extraction (*38*). Moreover, with the breakdown of the cellular constituents, bound phenolics compounds may be released (*8*).

Table 2.4: Effect of canning, storage temperature, and storage time on total anthocyanins, % polymeric color, total phenolics, ORAC, and FRAP of pitted Bing cherries and canned Bing cherries in light syrup

Samples	Storage Temperature (°C)	Storage Time (month)	Anthocyanins (mg/100g fw)	% Polymeric	Total Phenolic (mg/g fw)	ORAC (μmoles/g fw)	FRAP (μmoles/g fw)
Fresh		0	63.7 ± 0.9A	12.5 ± 2.3	1.94± 0.17B	13.12 ± 1.22B	14.32 ± 0.39 B
Total[a]	2	0	64.4 ± 1.3A**a**		2.59 ± 0.12A**a**	17.42 ± 2.91A**b**	19.97 ± 0.53A**a**
Cherry			35.1 ± 1.1	38.3 ± 5.7	1.17 ± 0.02	8.92 ± 1.78	9.59 ± 0.12
Syrup			29.9 ± 0.6	41.7 ± 2.3	1.41 ± 0.11	8.51 ± 1.16	10.38 ± 0.55
Total[a]	2	5	57.0 ± 5.4**a**		2.35 ± 0.07**a**	29.57 ± 1.42**a**	20.59 ± 0.92**a**
Cherry			28.0 ± 3.0	29.5 ± 4.6	1.27 ± 0.04	14.18 ± 1.75	10.07 ± 0.69
Syrup			29.0 ± 2.7	30.5 ± 2.5	1.08 ± 0.04	15.38 ± 0.44	10.52 ± 0.58
Total[a]	22	0	68.9 ± 6.9A**a**		2.33 ± 0.23A**a**	18.45 ± 1.28A**b**	18.55 ± 2.14A**b**
Cherry			33.3 ± 5.2	47.7 ± 5.8	1.13 ± 0.19	8.98 ± 1.02	8.79 ± 0.62
Syrup			35.6 ± 1.7	33.3 ± 3.8	1.20 ± 0.13	9.47 ± 0.49	9.75 ± 1.56
Total[a]	22	5	39.7 ± 1.2**b**		2.31 ± 0.06**a**	28.96 ± 0.89**a**	19.03 ± 0.43**b**
Cherry			19.7 ± 0.5	39.5 ± 1.8	1.21 ± 0.03	12.76 ± 0.86	8.98 ± 0.07
Syrup			20.0 ± 1.3	34.6 ± 3.8	1.10 ± 0.05	16.20 ± 0.17	10.02 ± 0.43

Values are mean ± standard deviations, n=2. Different letters within the same column indicate significantly different at $p \leq 0.05$.
[a]Values combine between cherry and syrup value.

Figure 2.15: Effect of canning and storage temperature and time on anthocyanin content of pitted Bing cherries and canned Bing cherries in light syrup (mg cyanidin-3-glucoside/100g, n =2)

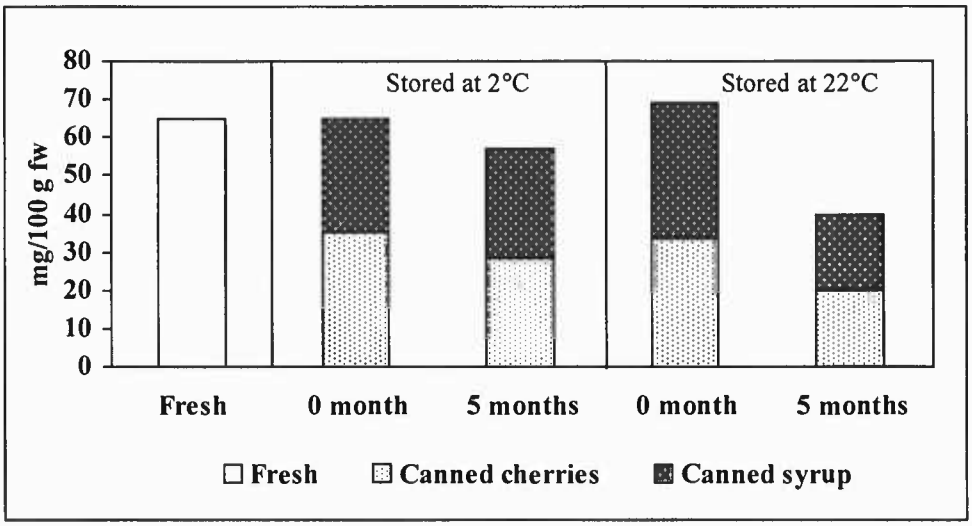


Figure 2.16: Effect of canning and storage temperature and time on total phenolics of pitted Bing cherries and canned Bing cherries in light syrup (mg gallic acid equivalent/g, n=2)

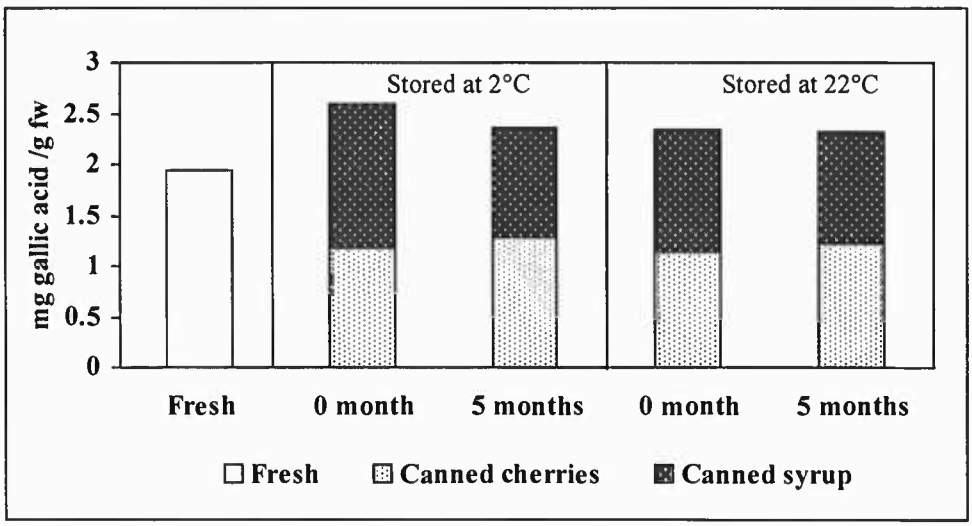


Figure 2.17: Effect of canning and storage temperature and time on ORAC of pitted Bing cherries and canned Bing cherries in light syrup (μmoles Trolox equivalent/g, n=2)

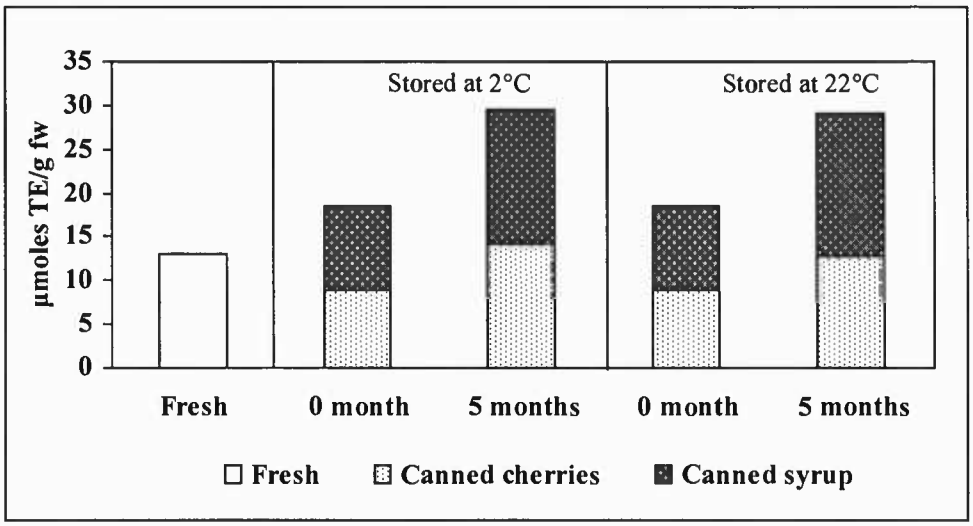
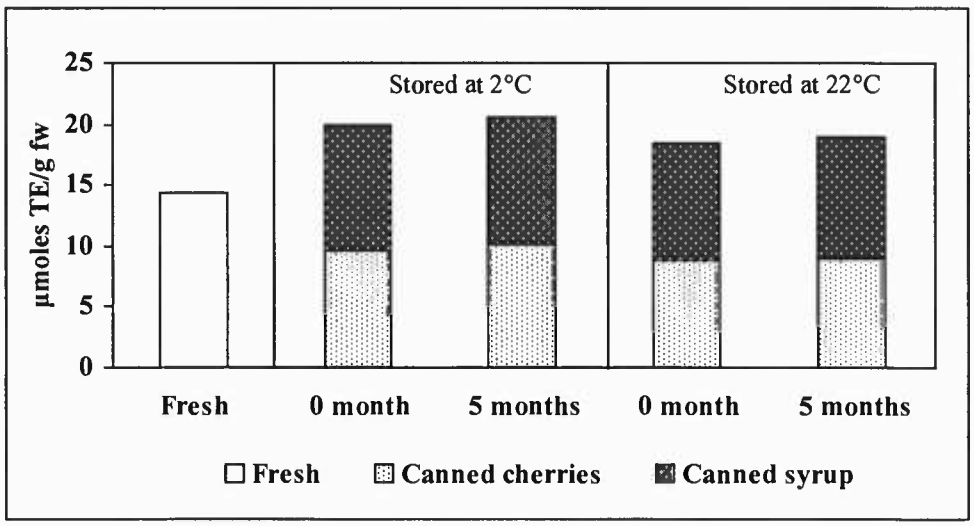


Figure 2.18: Effect of canning and storage temperature and time on FRAP of pitted Bing cherries and canned Bing cherries in light syrup (μmoles Trolox equivalent/g, n=2)



Kalt et al. (*21*) reported that extraction of blueberries at 60 °C resulted in higher total phenolics and antioxidant activities. Anese et al. (*3*) reported that heating increase the antioxidant potential in tomato juice as the consequence of the formation of Maillard reaction products.

The increase of % polymeric color in syrup and fruits with canning indicated that anthocyanin degradation occurred with canning. Polymeric color could also be formed from polyphenoloxidase activity before its inhibition. After 5 month storage at 22 °C, a significant ($p \leq 0.05$) decrease in total anthocyanins was observed while there was no significant decrease in total phenolics at either 2 or 22 °C. There was a substantial increase in antioxidant activities with storage at both temperatures. Samples stored at 2 °C had higher FRAP values than those stored at 22 °C while ORAC values increased after 5 month storage at either 2 or 22 °C. One possible explanation is the formation of Maillard browning reaction products from reducing sugars and amino acids. Several workers have reported that Maillard products and their intermediates possess antioxidant properties (*3, 4, 10, 24, 28*).

Effect of brining

Changes of total anthocyanins, total phenolics, ORAC, and FRAP in Bing and Royal Ann cherries after brining are presented in Table 2.5 and Figures 2.18-2.21. Little anthocyanins or phenolics remained in the cherries after brining and subsequent cold water washing. Water washing of bisulfite-brined cherries to reduce free SO_2 levels is a necessary unit operation before the cherries are manufactured into maraschino cherries, glaced cherries or other food products.

Roughly 50% of the anthocyanins and total phenolics are leached from the cherries into the brine solution. Anthocyanin losses of Bing and Royal Ann cherries were 57.5% and 84.1%, respectively, while total phenolics losses of were 91.1% and 50.8%. The spent brine is rich in antioxidant activity, presumably because of the anthocyanins and polyphenolics. With respect to the two cherry cultivars, Royal Ann brine was higher in antioxidant activity than the brine from Bing cherries. Royal Ann brine was also higher in total phenolics while Bing brine was higher in anthocyanin content. Spent brine solution is a processing waste that may be a useful source of anthocyanin pigments, polyphenolics, and antioxidants.

Table 2.5: Effect of brining on total anthocyanins, total phenolics, ORAC, and FRAP of pitted Bing and Royal Ann cherries and their brine solution

Cultivar	Samples	Processing	Anthocyanins (mg/100g fw)	Total Phenolic (mg/g fw)	ORAC (μmoles/g fw)	FRAP (μmoles/g fw)
Bing	Cherry	Before brining	26.1 ± 1.2A	1.8 ± 0.0B	13.0 ± 0.2A	11.5 ± 0.6B
		After brining	0.5 ± 0.02B	0.2 ± 0.0C	0.7 ± 0.03B	0.7 ± 0.0C
	Brined Solution	Before brining	0.0 ± 0.0b	1.38 ± 0.4c	1.19 ± 0.32c	11.84 ± 0.06b
		After brining	11.1 ± 0.7a	1.54 ± 0.0b	8.99 ± 0.77b	12.69 ± 1.7b
		After brining[a]	11.1 ± 0.7	0.16 ± 0.0	7.79 ± 0.8	0.14 ± 0.30
Royal Ann	Cherry	Before brining	0.63 ± 0.04B	2.4 ± 0.01A	14.3 ± 2.4A	15.5± 0.5A
		After brining	0.1 ± 0.03B	0.2 ± 0.01C	0.5 ± 0.03B	0.8 ± 0.1C
	Brined Solution	Before brining	0.0 ± 0.0b	1.38 ± 0.4c	1.19 ± 0.32c	11.84 ± 0.06b
		After brining	0.1 ± 0.0b	2.56 ± 0.04a	14.78 ± 0.36a	22.25 ± 0.19a
		After brining[a]	0.1 ± 0.0	1.18± 0.04	13.6 ± 0.37	10.4 ± 0.19

Values are mean ± standard deviations, n=2. Different letters within the same column indicate significantly different at $p \leq 0.05$. Due to the interference of brine solution with the determination of total phenolics, ORAC, and FRAP,[a] Values are subtracted by fresh brined solution (blank).

Figure 2.19: Effect of brining on anthocyanin content of pitted Bing and Royal Ann cherries and their brine solution (mg cyanidin-3-glucoside/100g, n=2)

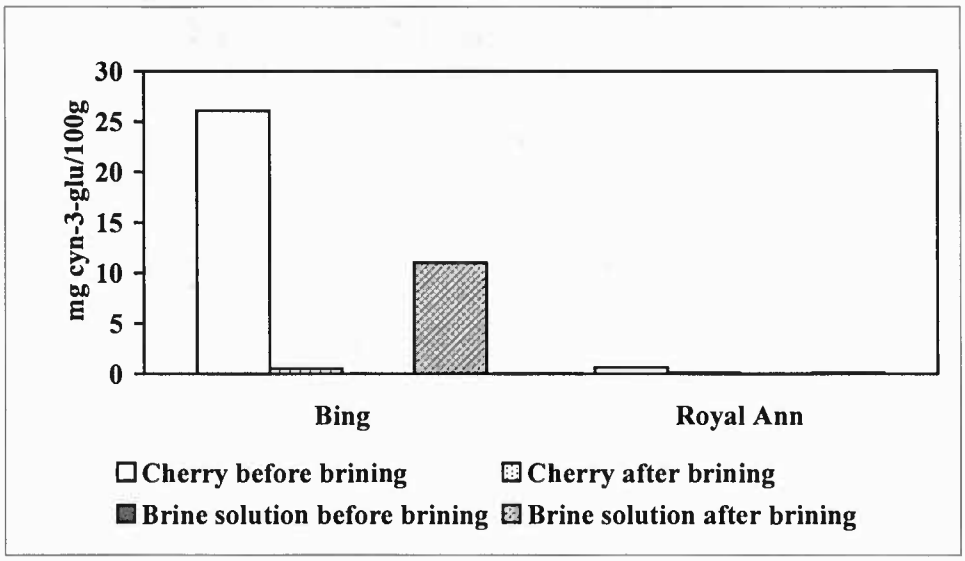


Figure 2.20: Effect of brining on total phenolics of pitted Bing and Royal Ann cherries and their brine solution (mg gallic acid equivalent/g, n=2)

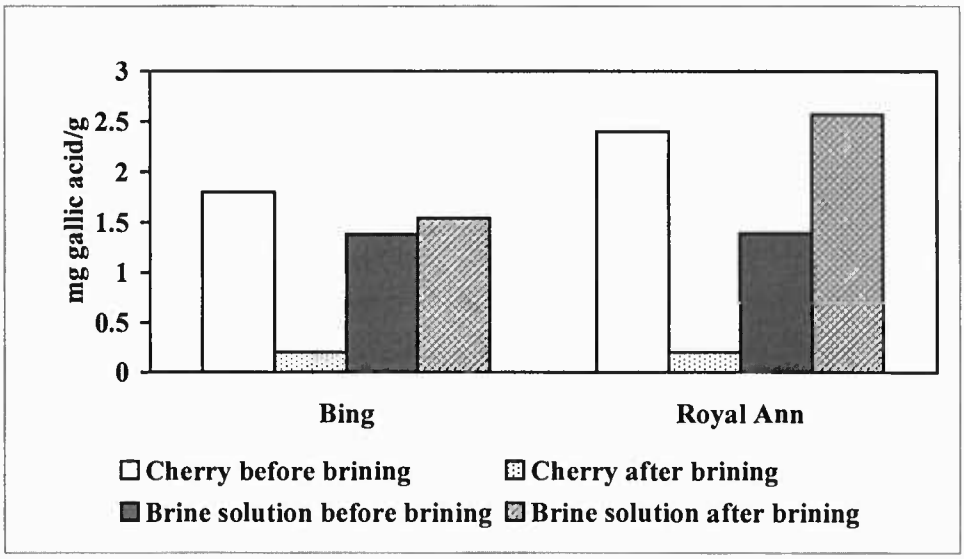


Figure 2.21: Effect of brining on ORAC of pitted Bing and Royal Ann cherries and their brine solution (μmoles Trolox equivalent/g, n=2)

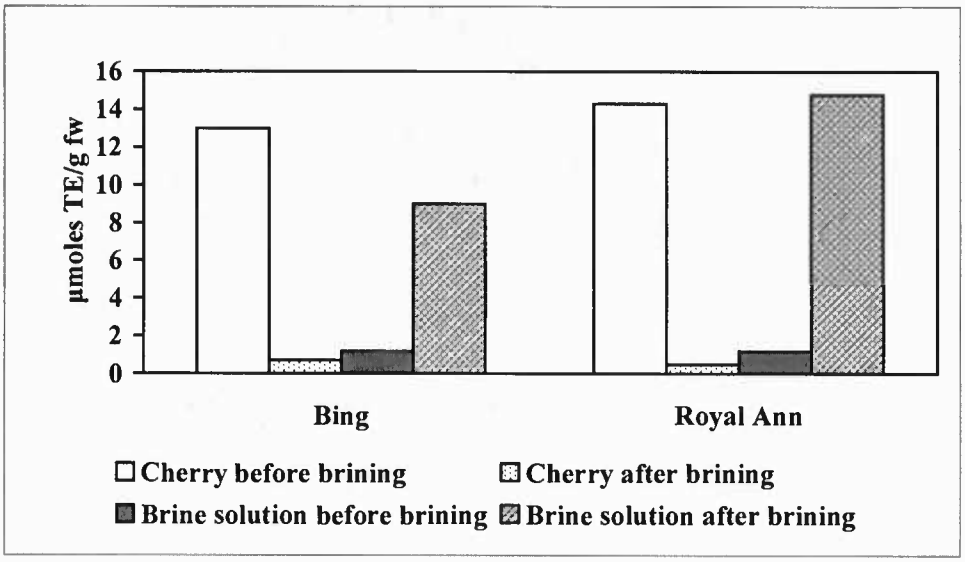
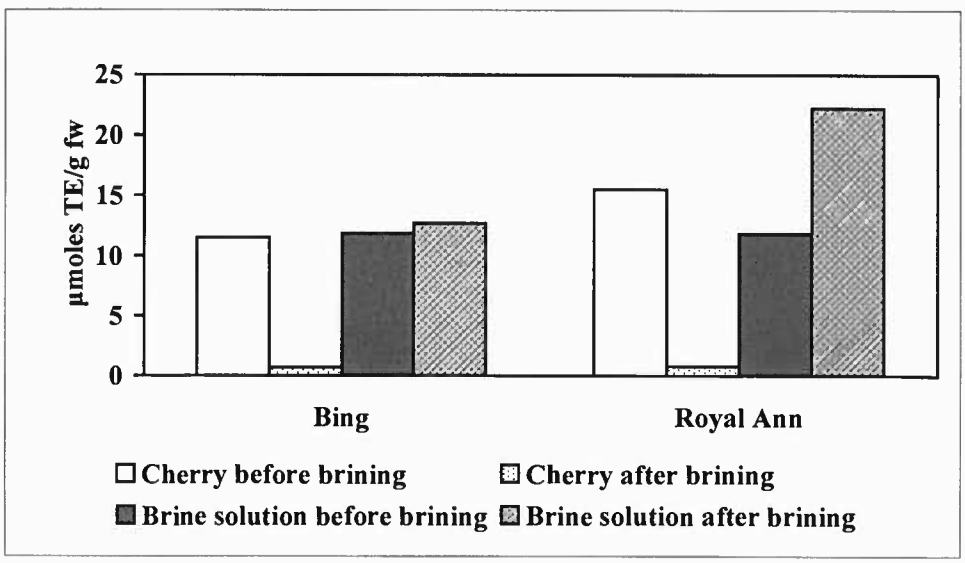


Figure 2.22: Effect of brining on FRAP of pitted Bing and Royal Ann cherries and their brine solution (μmoles Trolox equivalent/g, n=2)



CONCLUSIONS

Antioxidant activities were highly correlated with total phenolics. The highest antioxidant activity was for Montmorency cherries, with decreasing values for Bing, Royal Ann, and Rainier cherries. Frozen storage and canned storage had a more pronounced effect on anthocyanins than total phenolics. There was marked destruction of anthocyanins during frozen storage at –23 °C, but they were relatively stable at –70 °C storage. There was little loss of total anthocyanins with canning, but approximately 50% of anthocyanins and polyphenolics were redistributed to the syrup. Roughly half of the polyphenolics and anthocyanins were leached into the brine solution. Washing of the brined cherries to lower SO_2 levels resulted in removal of essentially all the anthocyanins and polyphenolics from the brined cherries. Cherry processing wastes may be potential sources for natural colorants, nutraceuticals and natural antioxidants.

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CHAPTER IV

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 HPLC with diode array detection (DAD). Changes during frozen storage, canning, and brining were also monitored. Cyanidin-3-rutinoside and cyanidin-3-glucoside are the major pigments in sweet cherries with an additional 5 minor pigments being identified; cyanidin-3-glucosylrutinoside and cyanidin-3-rutinoside are the major pigments in Montmorency cherries with an additional 4 minor pigments being present. Hydroxycinnamates are the major class of polyphenolics in sweet cherries (70-80%) while flavanols predominate in sour cherries (49.9%). Skins contained the highest concentrations of anthocyanins and polyphenolics. Flavonol glycosides are nearly exclusively found in skins. Hydroxycinnamates were greatly affected by processing and storage while flavonol glycosides were quite stable. Approximately half of the anthocyanins and polyphenolics were transferred to the syrup with canning, and nearly all were transferred to the brine during the brining process. Flavonol glycosides and hydroxycinnamates were not present in cherry brine while new compounds believed to be flavanol derivatives were detected.

Keywords: cherries, anthocyanins, polyphenolics, canning, brining, frozen storage, and HPLC-DAD

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 (*1, 6, 8, 23, 28, 40*). Heinonen et al. (*24*) compared the *in vitro* antioxidant activities of several different fruits and ranked sweet cherries (Bing and Burlat cultivars) first and third in activity. Our laboratory has recently investigated the anthocyanin pigment and total phenolic contents of cherries along with their antioxidant properties (*12*). The antioxidant properties of sour cherries have been extensively investigated by Michigan workers (*47, 55, 58*).

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 (*22, 34, 36*). Cyanidin-3-glucosylrutinoside, cyanidin-3-rutinoside, cyanidin-3-glucoside, and cyanidin-3-sophoroside have been identified in sour cherries (*10, 13*); cyanidin-3-arabinosylrutinoside, cyanidin-3-gentiobioside, and peonidin-3- rutinoside have also been reported to be present (*11, 13, 46, 48*).

Hydroxycinnamates are a major class of polyphenolics that are present in cherries. Schaller and Von Elbe (*45*) identified six isomers of caffeoylquinic acid,

four 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 (*21, 22, 36*). The presence of caffeoyltartatric acid in sweet and sour cherries and 4'-*p*-coumaroylquinic acid in sour cherries has also been reported (*21*). 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 (*25, 45, 49*). In addition, Shrikhande and Francis (*49*) reported the presence of quercetin-4'glucoside, kaempferol-3-rhamnoside-4'-galactoside (tentative), and kaempferol-4'-glucoside (tentative). Henning and Herrmann (*25*) isolated the 3,4'-biglucosides 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 (*20, 21*) found the flavanol epicatechin in both sweet and sour cherries and procyanidins in sour cherries.

While all cherry cultivars have some anthocyanin pigmentation in the skins, the amounts vary tremendously (*12*). Some varieties have pigmentation in the flesh, e.g., Bing, and others do not, e.g., 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 frozen storage, canning, and brining (*12*). Approximately 75% of sweet cherry production are brined for subsequent manufacture into maraschino and glaced cherries; 12% are canned and 18% are frozen, dried and used for fruit juice (*15*). Processing clearly had an impact on all three 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 &METHODS

Standards

Phenolic standards (protocatechuic acid, chlorogenic acid, epicatechin, caffeic acid, *p*-coumaric acid, and quercetin-3-rutinoside) were purchased from Sigma Chemical Co. (St. Louis, MO). Quercetin-3-glucoside, quercetin-3-

rhamnoside, kaempfereol-3-glucoside, and kaempferol-3-rutinoside were purchased from Extrasynthese (Genay, France). Concord grape juice concentrate (Welch Food Inc, Concord, MA) was purchased at a local supermarket while cranberry, black currant, red raspberry, and strawberry juice concentrates were provided by Kerr Concentrates Inc (Salem, OR).

Sources of cherry samples

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

Fresh cherries

Cherry samples (250 g) were separated into skins, flesh, and pits by hand. 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 two replications.

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, closed, and stored at -23 °C and –70 °C.

Canned cherries

Stemmed Bing cherries (2.5 Kg) were washed, destemmed, and pitted using a hand cherry pitter. Pitted cherries were filled into cans No.303 with dark fruit enamel. Sucrose syrup (C&H Superfine sugar diluted to 19 °Brix with water, C&H Sugar Company, Inc., Crockett CA) was added and the filled cans were heated to a temperature of 80 °C. Cans were exhausted on a steam bath for 2 min 30 second, and sealed with #303 lids by Canco 006 Steam-flow Can seamer. Cans were

immersed in water and heated at 100 °C for 12 min. The canned fruits were cooled by placing in 25 °C water and then stored at 2 and 22 °C. The canning experiment was replicated twice.

Brined cherries

Royal Ann and Bing cherries with stems (about 1 Kg) were each placed in 4 L glass jars. The bisulfite brine was added to the fruit-containing jars until the fruits were covered. Bisulfite brine solution was prepared by dissolving 220 g of sodium metabisulfite, 202 g of calcium chloride, and 10 g citric acid in 10 L of water. This gave a solution, which was 2.2% sodium metabisulfite, 2% calcium chloride, and 1% citric acid. The pH was about 3.0. Plastic wrap covered the glass jars to allow for air diffusion. The brined fruit was stored for 12 months at 22 °C. After 12 months, cherries were washed with running cold water about 5 days until sulfur dioxide content of cherries was less than 200 ppm. Washed brined 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 (ca. 10 g) were blended with 20 mL of acetone, sonicated (Ultrasonic cleanser, Branson Cleaning Equipment Corp., Shelton, CT)

for 10 min and then filtered on a Büchner funnel using Whatman No.1 paper. 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 *Xg* for 20 min by IEC International Centrifuge (Model UV, International equipment Co., Boston, MA). The aqueous phase was collected and evaporated *in vacuo* at 40 °C until the residual acetone residual was removed (ca. 10 min). The fraction was made up to 25 mL with acidified water and stored at –70 °C until further analyzed. Sample extractions were replicated twice.

Anthocyanin and polyphenolic purification

The purification of anthocyanins and polyphenolics were conducted as described by Rodriguez-Saona and Wrolstad (*42*). The aqueous extract (ca. 2 mL) was passed through a C-18 Sep-Pack cartridge (Waters Assoc., Milford, MA), previously activated with methanol followed by 0.01% aqueous HCl (v/v).

Anthocyanins and polyphenolics were adsorbed onto the mini-column while sugars, acids, and other water-soluble compounds were removed by washing mini-column with 10 mL of 0.01% aqueous HCl (v/v). Then, the mini-column was dried with nitrogen gas. Polyphenolics were subsequently eluted with 5 mL of ethyl acetate. Anthocyanins were eluted with 5 mL of acidified methanol.

The ethyl acetate fraction containing the polyphenolics and the methanol fraction containing the anthocyanins were concentrated to dryness using a Büchi rotavapor-R (Brinkmann Instruments, Westbury, NY) at 40 °C. The polyphenolics

and anthocyanins were redissolved in acidified water and stored at –70 °C. Samples were filtered through a 0.45 μM Milipore filter paper (Type HA) before HPLC injection.

Acid hydrolysis of anthocyanins

Acid hydrolysis was performed by the procedure as described by Durst and Wrolstad (*14*). Five mL of 2N HCl were added to purified anthocyanins (ca. 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., Milford, MA) as previously described.

Alkaline hydrolysis of anthocyanins and polyphenolics

The saponification was performed by the procedure as described by Durst and Wrolstad (*14*). The anthocyanin or polyphenolic isolate (ca. 1mL) 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 2N HCl was added to neutralize the solution. The hydrolysate was purified by solid-phase extraction using a C-18 Sep-Pak cartridge (Waters Assoc., Milford, MA) as described above.

High Performance Liquid Chromatography analytical system

A high performance liquid chromatography 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 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 quantification. Samples were injected by Agilent 1100 Series auto sampler (Agilent Technologies Wilmington, DE). Injection volume for anthocyanin analysis was 50 μL while that for polyphenolic analysis was 20 μL. The flow rate was 1mL/minute.

HPLC separation of anthocyanins and saponified anthocyanins

Anthocyanins and saponified anthocyanins were separated using a Prodigy ODS-3 column (5 μm) 250 x 4.6 mm i.d. (Phenomenex, Terrance, CA), fitted with an Allsphere 10 x 4.6 mm i.d. ODS-2 guard column (Alltech, Deerfield, IL). Solvent A was 100% HPLC grade acetonitrile and solvent B was 1% phosphoric acid, 10% acetic acid (glacial), 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 utilized a Prodigy ODS-3 column (5 μm) 250 x 4.6 mm i.d. (Phenomenex, Terrance, CA), fitted with an Allsphere 10 x 4.6 mm i.d. ODS-2 guard column (Alltech, Deerfield, IL). Solvents A and B as described above 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 x 4.6 mm i.d. (Phenomenex, Terrance, CA), fitted with an Allsphere 10 x 4.6 mm i.d. ODS-2 guard column (Alltech, Deerfield, IL). Solvent A was 100% HPLC grade acetonitrile while solvent B was 1% acetic acid in deionized water. The program was isocratic at 5% A for 5 min, then 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 x 4.6 mm i.d. (Phenomenex, Terrance, CA), fitted with an Allsphere 10 x 4.6 mm i.d. ODS-2 guard column (Alltech, Deerfield, IL). Solvent A was 100% HPLC grade acetonitrile while Solvent B was 0.07M KH_2PO_4 adjusted to pH 2.4 with conc. H_3PO_4. 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 quercetin-3-rutinoside (rutin) at 260 nm.

Electrospray Mass Spectrometry (ESMS)

Low-resolution MS was obtained using ESMS. The instrument was a Perkin Elmer SCIEX API III bimolecular mass analyzer (Ontario, Canada) 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, WA). To assess the effect of frozen storage on anthocyanin peak area and individual phenolic content, a one factor ANOVA model was used, the factor being fresh cherries and the combination of frozen temperatures and storage time. To evaluate the effect of canning on individual phenolic content, a one factor ANOVA model was used with the levels of the factors as follows: fresh cherries and canned cherries at two levels of temperatures. To evaluate the effect of canned storage on individual phenolic content, a two factor ANOVA model was used with the factors as follows: time and temperature. The Pairwise comparison between treatment level means in all ANOVA models was carried out using Tukey's method ($p \leq 0.05$). To compare the mean of anthocyanin peak area in fresh cherries and brined cherries or spent brined solution for each cherry variety, a t-test was used to analyze separately. The Pearson correlation was used to determine the correlation among antioxidant activities and phenolic composition in skins, flesh, and pits of cherries.

RESULTS & DISCUSSION

Anthocyanin composition of cherries

Eight different anthocyanins were identified in sweet and sour cherries (Table 3.1). 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 (*51*); cyanidin-3-sophoroside and cyanidin-3-glucosylrutinoside from red raspberries (*52*); pelargonidin-3-glucoside and pelargonidin-3-rutinoside from strawberries (*9*); and peonidin-3-glucoside from cranberries (*27*). HPLC analysis of the acid hydrolysis products of the anthocyanin isolates showed cyanidin to be the major anthocyanidin 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 (*10, 13, 22, 33, 34, 36, 48, 56*) with the exception that this is the first report for the presence of pelargonidin-3-glucoside.

Table 3.1: Relative anthocyanin compositions of skins, flesh, and pits of four cherry cultivars: Bing, Royal Ann, Rainier, and Montmorency

Cultivar	Portion	ACN[a]	(% total peak area at 520 mn)							
			Cyanidin-3-sophoroside	Cyanidin-3-glucosylrutinoside	Cyanidin-3-glucoside	Cyanidin-3-rutinoside	Pelargonidin-3-glucoside	Pelargonidin-3-rutinoside	Peonidin-3-glucoside	Peonidin-3-rutinoside
Bing	Skins	60.6	nd	nd	18.99 ± 0.76	78.80 ± 0.96	0.33 ± 0.03	0.54 ± 0.03	0.23± 0.02	1.11 ± 0.20
	Flesh	26.0	nd	nd	36.72 ± 1.97	55.88 ± 2.43	0.34 ± 0.03	0.30 ± 0.03	1.34 ± 0.42	5.42 ± 0.20
	Pits	10.4	nd	nd	51.38 ± 2.43	30.81 ± 3.07	nd	nd	8.52 ± 0.75	9.29 ± 0.67
Royal Ann	Skins	2.2	3.42 ± 0.14	nd	3.34± 0.06	87.54 ± 0.04	nd	5.70 ± 0.24	nd	nd
	Flesh	0.1	nd	nd	nd	nd	nd	nd	nd	nd
	Pits	0.2	nd	nd	12.33 ± 0.73	59.12 ± 1.18	nd	nd	nd	28.55 ± 0.91
Rainier	Skins	2.1	1.17 ± 0.09	nd	2.51 ± 0.21	93.64 ± 0.21	nd	1.76 ± 0.08	nd	0.92 ± 0.05
	Flesh	0.0	nd	nd	nd	nd	nd	nd	nd	nd
	Pits	0.1	nd	nd	8.13 ± 0.54	56.18 ± 1.85	nd	nd	nd	35.69 ± 1.75
Montm-orency	Skins	36.5	0.97 ± 0.03	62.96 ± 0.83	1.65 ± 0.10	28.65 ± 0.53	0.89 ± 0.02	nd	nd	4.88 ± 0.21
	Flesh	0.0	nd	nd	nd	nd	nd	nd	nd	nd
	Pits	0.8	12.97 ± 1.06	61.69 ± 0.81	10.50 ± 0.73	11.49 ± 0.76	1.55 ± 0.12	nd	nd	1.80 ± 0.20

[a] Anthocyanin content were reported as mg cyanidin-3-glucoside/100g fresh weight (fw) (*12*).
Each value in table is mean ± standard deviation (n=2).

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 second largest pigment. This is consistent with previous reports (*11, 22, 26, 36*). The three 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 first report on the anthocyanin composition of Rainier and Royal Ann cherries.

Gao and Mazza (*22*) and Mozetič et al. (*36*) 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 (*22*). Sour cherries contained cyanidin-3-sophoroside and cyanidin-3-glucosylrutinoside along with most anthocyanins present in sweet cherries except for pelargonidin-3-rutinoside (*10, 13, 26*). Cyanidin-3-arabinosylrutinoside has been found in Balaton cherries (*11*).

Bing is the only cultivar containing anthocyanins in skins, flesh, and pits, the skins being richest in anthocyanin concentration (Table 3.1). Figure 3.1 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 (ca. 79% and 56%), while the major anthocyanin in Bing pits

was cyanidin-3-glucoside (ca. 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 while there is no presence of cyanidin-3-sophoroside in Rainier and Royal Ann pits. Figures 3.2-3.4 compare the HPLC anthocyanin profiles of skins and pits in Royal Ann, Rainier, and Montmorency cherries.

Figure 3.1: HPLC anthocyanin profile of Bing skins (A), flesh (B), and pits (C) at 520 nm. 1: cyanidin-3-glucoside, 2: cyanidin-3-rutinoside, 3: pelargonidin-3-glucoside, 4: pelargonidin-3-rutinoside, 5: peonidin-3-glucoside, 6: peonidin-3-rutinoside

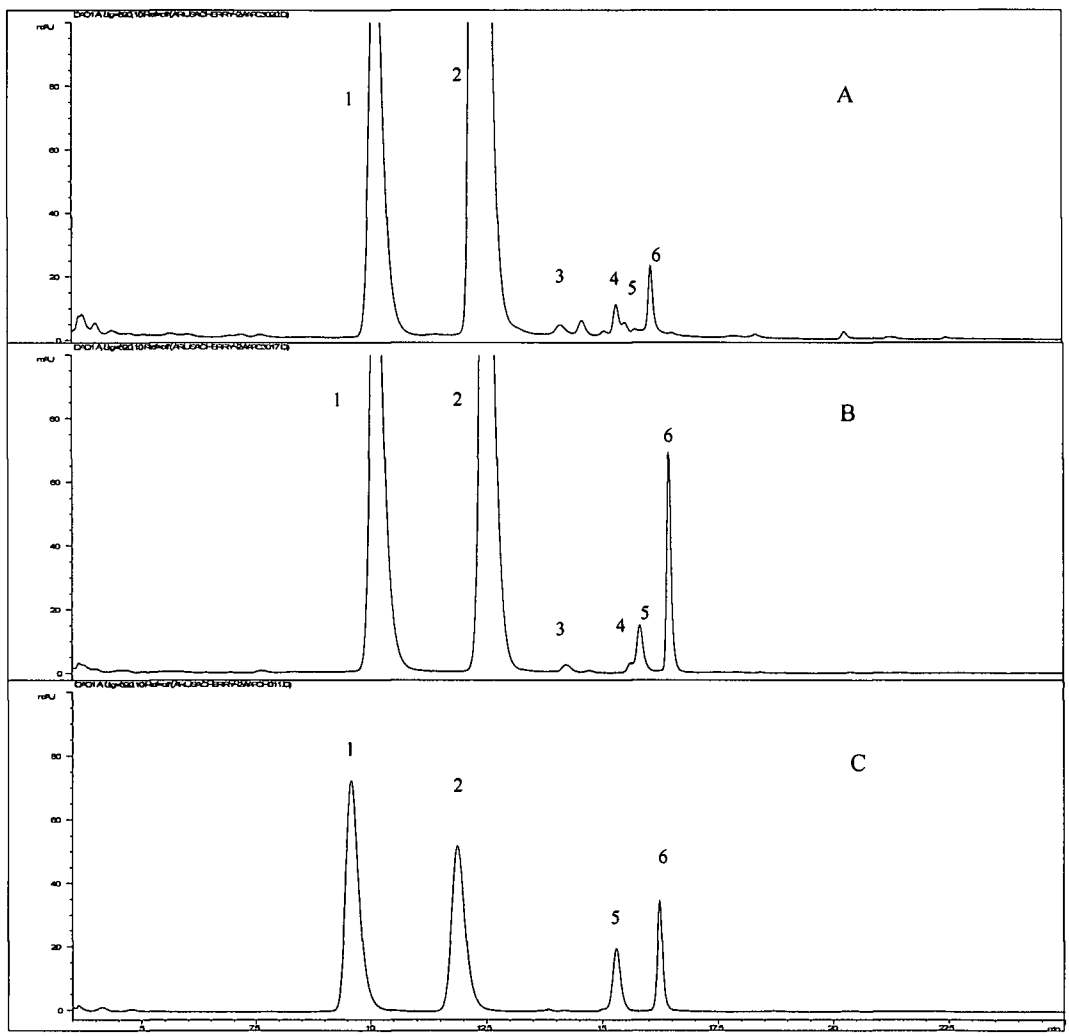


Figure 3.2: HPLC anthocyanin profile of Royal Ann skins (A) and pits (B) at 520 nm. 1: cyanidin-3-sophoroside, 2: cyanidin-3-glucoside, 3: cyanidin-3-rutinoside, 4: pelargonidin-3-rutinoside, 5: peonidin-3-rutinoside

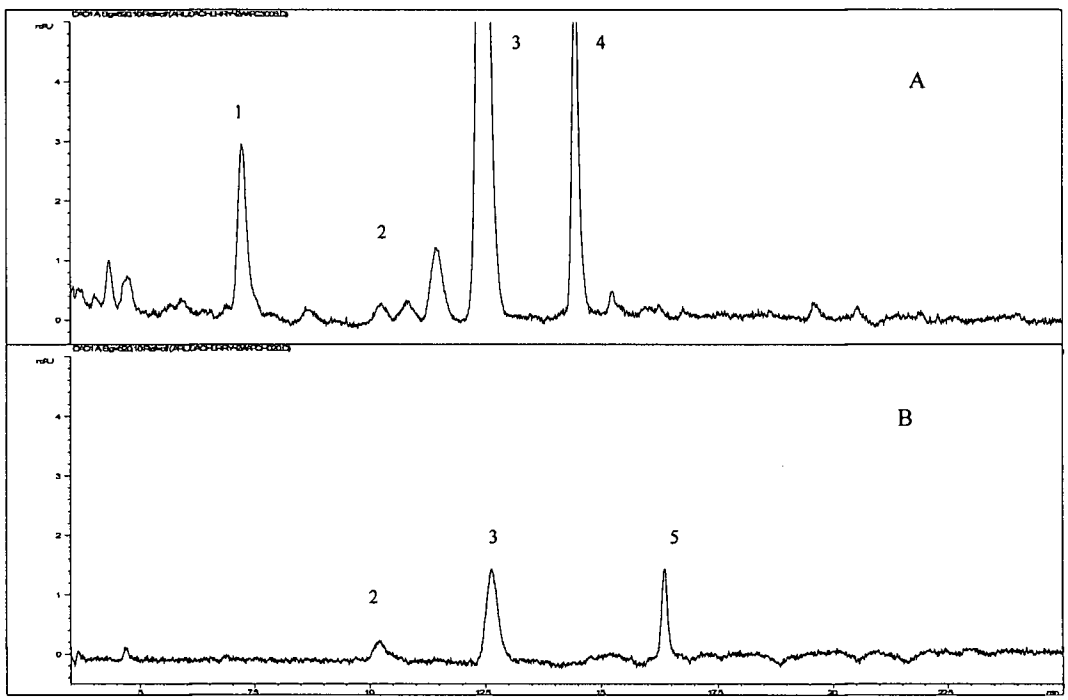


Figure 3.3: HPLC anthocyanin profile of Rainier skins (A) and pits (B) at 520 nm. 1: cyanidin-3-sophoroside, 2: cyanidin-3-glucoside, 3: cyanidin-3-rutinoside, 4: pelargonidin-3-rutinoside, 5: peonidin-3-rutinoside

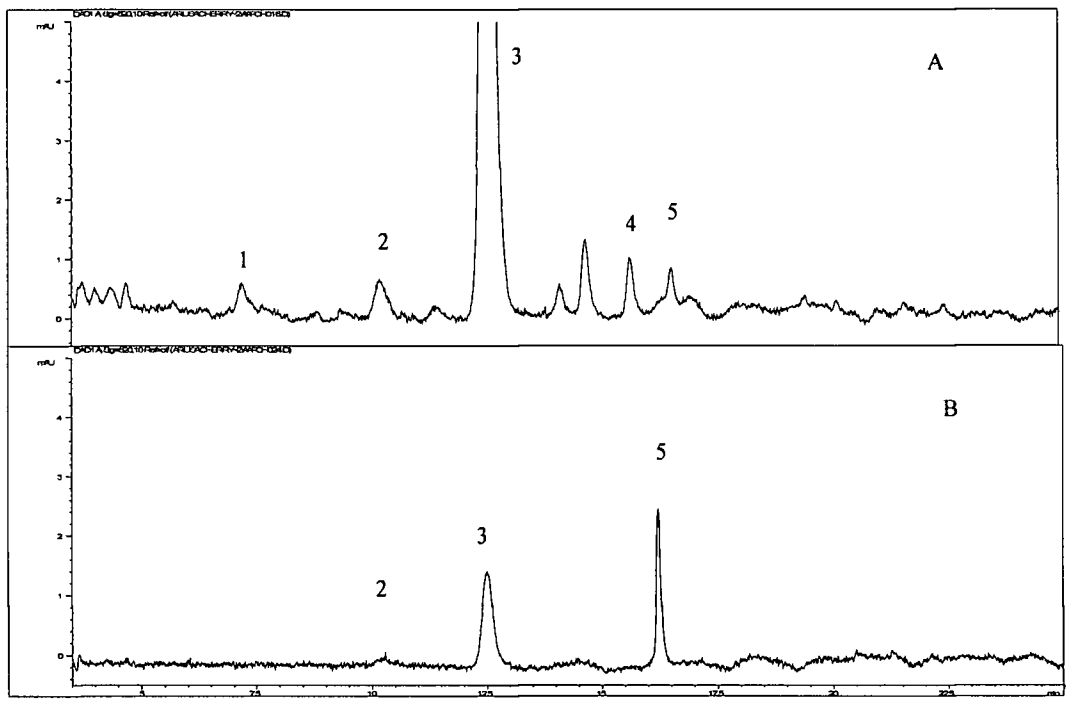
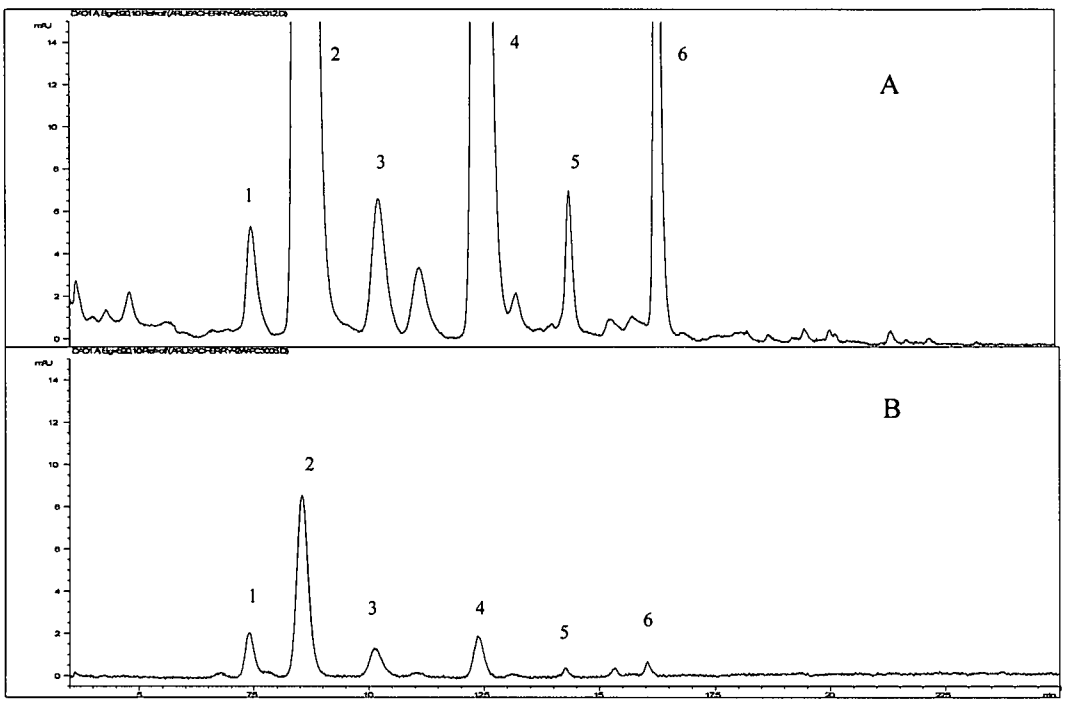


Figure 3.4: HPLC anthocyanin profile of Montmorency skins (A) and pits (B) at 520 nm. 1: cyanidin-3-sophoroside, 2: cyanidin-3-glucosylrutinoside, 3:cyanidin-3-glucoside, 4: cyanidin-3-rutinoside, 5: pelargonidin-3-glucoside, 6: peonidin-3-rutinoside



Polyphenolic composition of cherries

The polyphenolics were separated from the anthocyanins by solid-phase extraction on C-18 resin using ethyl acetate solvent. 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 Figures 3.5-3.8 showing HPLC chromatograms of Bing, Royal Ann, Rainier, 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, e.g. 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 Elecrospray mass spectra. These peak assignments are consistent with previous identifications reported in the literature (*21, 22, 36, 45, 54, 57*).

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 3.2 compares amounts of the different polyphenolics for skins, flesh and pits of the 4 different cultivars. The polyphenolic content for the edible portion

Figure 3.5: HPLC polyphenolic profile of Bing skins (A), flesh (B), and pits (C) at 260 nm, 280 nm, 320 nm, 370 nm, and 520 nm. 1: neochlorogenic acid, 2: 3'-*p*-coumaroylquinic acid, 3: chlorogenic acid, 4: epicatechin, 5: quercetin-3-rutinoside, 6: quercetin-3-glucoside, 7: kaempferol-3-rutinoside, HC: unidentified hydroxycinnamates, A: anthocyanins

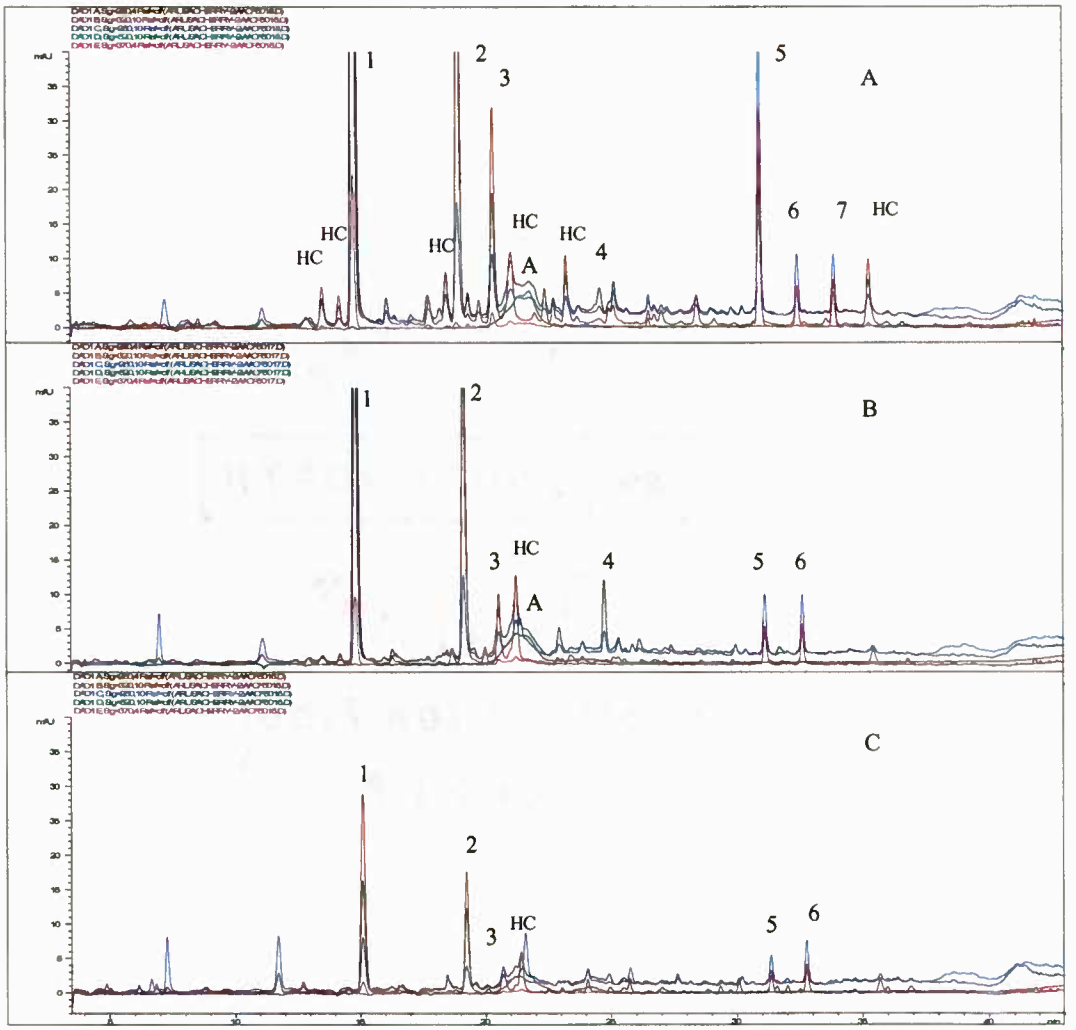


Figure 3.6: HPLC polyphenolic profile of Royal Ann skins (A), flesh (B), and pits (C) at 260 nm, 280 nm, 320 nm, 370 nm, and 520 nm. 1: neochlorogenic acid 2: 3'-*p*-coumaroylquinic acid, 3: chlorogenic acid, 4: epicatechin, 5: quercetin-3-rutinoside, 6: kaempferol-3-rutinoside, HC: unidentified hydroxycinnamates, P: unidentified flavanols, F: unidentified flavonol glycosides

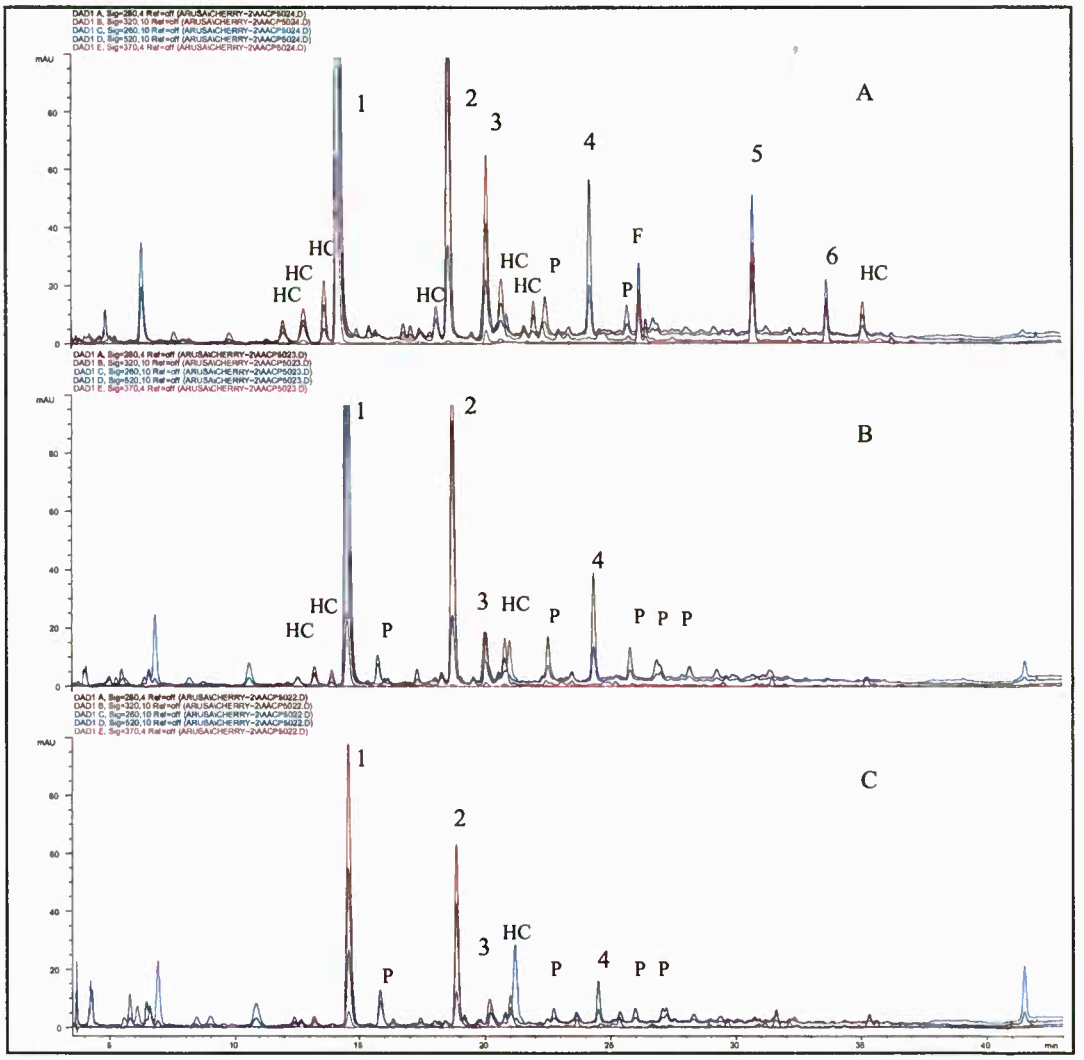


Figure 3.7: HPLC polyphenolic profile of Rainier skins (A), flesh (B), and pits (C) at 260 nm, 280 nm, 320 nm, 370 nm, and 520 nm. 1: neochlorogenic acid, 2: 3'-*p*-coumaroylquinic acid, 3: chlorogenic acid, 4: epicatechin, 5: quercetin-3-rutinoside, 6: kaempferol-3-rutinoside, HC: unidentified hydroxycinnamates, P: unidentified flavanols, F: unidentified flavonol glycosides

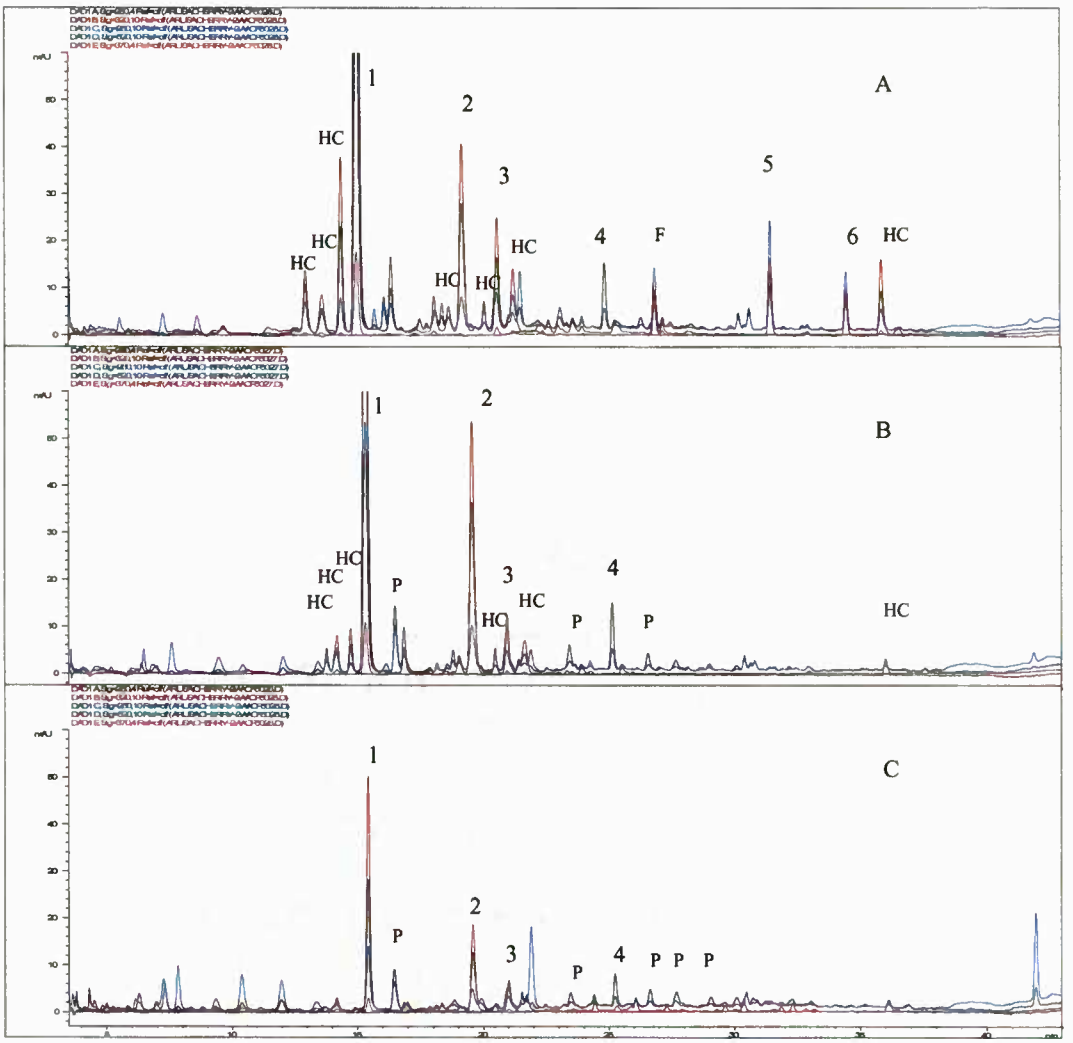


Figure 3.8: HPLC polyphenolic profile of Montmorency skins (A), flesh (B), and pits (C) at 260 nm, 280 nm, 320 nm, 370 nm, and 520 nm. 1: neochlorogenic acid, 2: 3'-*p*-coumaroylquinic acid, 3: chlorogenic acid, 4: epicatechin, 5: quercetin-3-rutinoside, 6: quercetin-3-glucoside, 7: kaempferol-3-rutinoside, HC: unidentified hydroxycinnamates, P: unidentified flavanols, F: unidentified flavonols

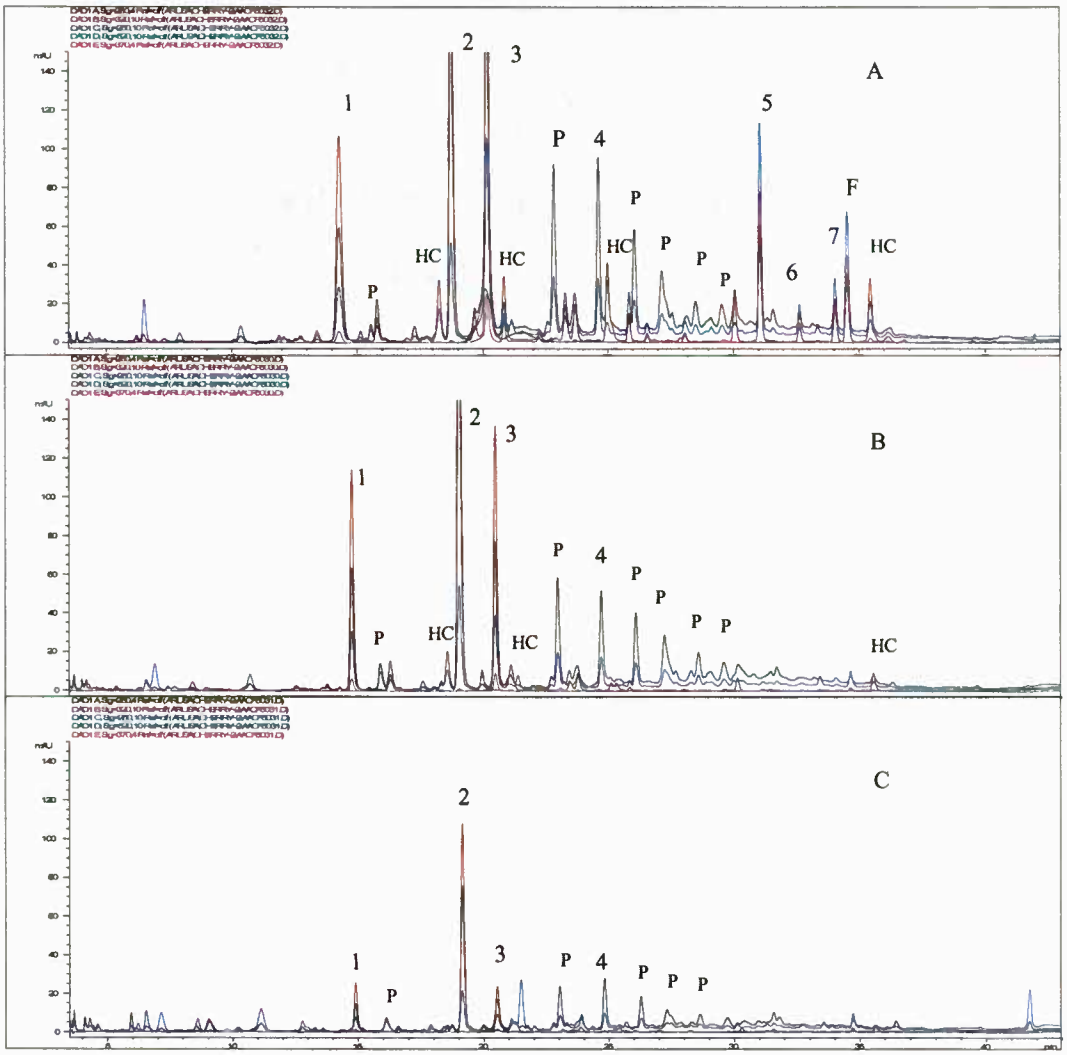


Table 3.2: Total phenolic content, % area quantified, hydroxycinnamates, epicatechin, procyanidins, flavonol glycosides, ORAC, and FRAP in skins, flesh, and pits of sweet cherries and sour cherries

[a] Amount in the edible portion (skins plus flesh) was calculated from the proportionate weight of skins and flesh except for total phenolics, ORAC, and FRAP values which were reported in a previous publication (*12*).
[b] Total phenolics were reported as mg gallic acid /100g fw.
[c] % Area denotes the percentage of the total peak area that was measured, e.g., unidentified peaks accounts for 6.9% of the total peak area for Bing flesh.
[d] ORAC and FRAP were reported as μmoles Trolox equivalent/100g fw.
Each value in table represents mean ± standard deviation (n=2).

Table 3.2: Total phenolic content, % area quantified, hydroxycinnamates, epicatechin, procyanidins, flavonol glycosides, ORAC and FRAP in skins, flesh, and pits of sweet cherries and sour cherries

cultivar	portion	TP [b]	% Area [c]	Hydroxycinnamates (mg chlorogenic acid /100g)	Epicatechin (mg epicatechin /100g)	Procyanidins (mg epicatechin /100g)	Flavonol glycosides (mg rutin/100g)	ORAC [d]	FRAP [d]
Bing	Edible [a]	185.0		56.8	8.29	nd	4.06	1494	1590
	Skins	333	99.6± 0.03	144.51 ± 7.21	18.54±1.07	nd	17.23±1.51	2826	2105
	Flesh	134	93.1± 0.9	48.16± 6.03	7.71± 0.70	nd	1.59±0.21	907	728
	Pits	92	65.2 ± 3.5	8.53 ± 2.02	2.51±0.49	4.70±0.81	nd	594	504
Royal Ann	Edible [a]	229		87.0	11.9	20.2	3.72	1449	1553
	Skins	351	98.4± 0.3	196.15± 11.79	19.61±2.49	24.61± 2.75	20.64±3.19	2744	1708
	Flesh	176	97.0 ± 0.3	83.04 ± 1.02	13.46±0.52	25.32±1.13	nd	1310	903
	Pits	104	63.2 ± 8.5	14.45 ± 1.28	2.70±0.25	7.34±0.88	nd	568	498
Rainier	Edible [a]	75		30	3.35	7.2	1.15	498	292
	Skins	142	93.8 ± 3.1	72.12 ± 2.63	4.70±0.75	0.97± 0.27	8.49±0.96	1050	592
	Flesh	65	90.3 ± 0.8	28.42 ± 0.37	3.77±0.44	9.90± 0.44	nd	462	227
	Pits	54	68.1 ± 0.8	5.58 ± 0.38	1.22±0.17	4.83±0.56	nd	338	200
Montm orency	Edible [a]	407		58.2	19.6	49.5	11.2	2557	3756
	Skins	558	98.4 ± 0.1	139.02 ± 2.24	50.47±2.61	108.07±4.00	63.22±4.17	5102	4796
	Flesh	301	97.1 ± 1.3	55.66 ± 0.45	17.74±1.25	50.30±5.13	nd	1500	1381
	Pits	157	70.6 ± 0.7	12.67 ± 0.23	8.15±0.38	22.52±1.86	nd	997	848

(skins 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 non-anthocyanin polyphenolics in Bing skins represent 99.6% of the total peak area, whereas the non-anthocyanin 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 hydroxycinnamate peaks, 1 flavanol peak, and 2 flavonol glycoside peaks (Figures 3.5-3.7). Bing contained in addition 1 flavonol glycoside peak while Royal Ann and Rainier contained 1 more hydroxycinnamate peak. 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 (*20, 21, 22, 36*). Gao and Mazza (*22*) and Mozetič et al. (*36*) reported that the major phenolic of sweet cherries was neochlorogenic acid while Friedrich and Lee (*21*) reported that the major phenolic in sweet and sour cherries was caffeoyltartaric acid, which was not evident from ESMS mass spectra data in our investigation. The remaining six peaks with spectra characteristic of hydroxycinnamates were measured as hydroxycinnamates.

Skins contained the highest concentrations of hydroxycinnamates, with substantially less in the flesh (Table 3.2). Note that the amounts in the edible portion were slightly greater than in flesh by itself since the skins are a relatively small proportion of the fruits. While pits were low in hydroxycinnamates compared with skins and flesh, they still accounted for a substantial proportion of the polyphenolics in sweet cherry pits. Of the quantified polyphenolics, the major hydroxycinnamates in sweet cherry skins, flesh, and pits were neochlorogenic acid (54-57%, 42-56%, and 25-31%) 3'-*p*-coumaroylquinic acid (6-10%, 13-18%, and 7-19%), and chlorogenic acid (4-5%, 1-12%, and 1.1-1.4%).

Montmorency skins contained 7 hydroxycinnamate peaks, 6 flavanol peaks, and 4 flavonol glycoside peaks (Figure 3.8), 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 (*21, 45*). The major hydroxycinnamates in sour cherry skins, flesh, and pits were different. Of the quantified 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 spectra and retention time with an authentic standard. It was present in all cultivars. The presence of epicatechin was in agreement with others (*5, 21*). 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 flesh alone.

Procyanidins were identified by matching UV spectra with epicatechin (*7*). There were large cultivar differences with respect to procyanidins. No procyanidins were detected in Bing skins and flesh while 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 (29-52.5%). Procyanidin B2 has been identified in sour cherries (*20, 21*).

Three flavonol glycoside peaks in sweet and sour cherries were identified as quercetin-3-rutinoside, quercetin-3-glucoside, and kaempferol-3-rutinoside, which are in agreement with Shrikhande and Francis (*49*), Schaller and Von Elbe (*45*), and Wang et al. (*57*). Quercetin-3-rutinoside and kampferol-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 Herrman (*25*) also reported that the glucosides 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 (*45, 57*), 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 spectra. 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 3.2). The flavonol glycoside concentration in Bing flesh was relatively low.

While the qualitative composition of sweet and sour cherries are similar (Figures 3.5-3.8), the proportions of polyphenolics are different (Table 3.2). In the edible portion (skins plus flesh), major compounds of sweet cherries were hydroxycinnamates (70.7-82.1%), epicatechin (8.0-12%), and flavonol glycosides (2.8-5.9%). 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/100g), procyanidins (49.5 mg/100g), and flavonol glycosides (11.2 mg/100g), and Royal Ann cherries contained highest content of hydroxycinnamates (87 mg/100g). Within sweet cherries, Bing cherries contained the highest content of flavonol glycosides (4.1 mg/100g) while Royal Ann cherries contained the highest epicatechin (11.9 mg/100g) and procyanidins (20.2 mg/100g). 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 3.2) and total anthocyanins (Table 3.1); e.g. total phenolics of Bing skin are 333 mg/100 g fw whereas the sum of total polyphenolic classes and anthocyanins are 240.88 mg/100g. This is not surprising as 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 two different procedures (r=0.97).

In this investigation, Bing cherries were used for processing experiments. The polyphenolic composition of the different lots of Bing cherries is shown in Tables 3.4 and 3.6. The two different lots havr similar proportions of different polyphenolics, but there is substantial difference in individual levels, e.g. 57 vs 74 mg/100 g of hydroxycinnamates. The levels of hydroxycinnamates which we measured in Bing cherries are much lower than values reported in Gao and Mazza (*22*), but higher than the amounts reported by Mozetič et al. (*36*). 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

Tables 3.3 and 3.4 compare the anthocyanin and polyphenolic composition of fresh and frozen Bing cherries stored at–23 °C and –70 °C for 3 and 6 months. There was a pronounced loss of total anthocyanins during storage at –23 °C, 67% after 3 months and 88% after 6 months. Losses were much less at –70 °C, 11% and 12% after 3 and 6 months, respectively. Polesello and Bonzini (*39*) reported 34-74% anthocyanin loss in sweet cherries after 4 month storage at –20 °C.

Table 3.3: Relative anthocyanin composition of fresh and frozen Bing cherries stored at 3 months and 6 months at 2 different temperatures (-23 °C and –70 °C)

	Tempt (°C)	Time (months)	ACN[a] (mg/100g)	Anthocyanins (% total peak area at 520 mn)					
				Cyanidin-3-glucoside	Cyanidin-3-rutinoside	Pelargonidin-3-glucoside	Unknown	Pelargonidin-3-rutinoside	Peonidin-3-rutinoside
Fresh			63.7	8.05 ± 0.08a	85.21 ± 0.17a	0.56 ± 0.03c	nd	0.91 ± 0.02b	5.26 ± 0.09cd
Freezing	-23	3	21.5	8.90 ± 0.76a	80.29 ± 0.77b	1.35 ± 0.03b	0.29 ± 0.06b	1.05 ± 0.15a	8.11 ± 0.17a
Freezing	-23	6	8.02	7.56 ± 0.07a	80.75 ± 0.44b	3.06 ± 0.17a	0.65 ± 0.02a	0.94 ± 0.08ab	7.05 ± 0.17b
Freezing	-70	3	57.4	9.40 ± 1.12a	84.55 ± 0.81a	0.43 ± 0.013c	nd	0.79 ± 0.001b	4.84 ± 0.33d
Freezing	-70	6	56.4	9.36 ± 1.74a	83.94 ± 1.82a	0.46 ± 0.01c	nd	0.78 ± 0.07b	5.46 ± 0.13c

[a] Anthocyanin contents were reported as mg cyanidin-3-glucoside/100g fw (*12*). Each value in table is mean ± standard deviation (n=2). The values with different letters in a column indicate a significant difference at $\alpha = 0.05$. Tempt is temperature.

Table 3.4: Total phenolic content, hydroxycinnamates, epicatechin, flavonol glycosides, ORAC, and FRAP in fresh and frozen Bing cherries stored at –23 °C and -70°C for 3 and 6 month storage

	Tempt (°C)	Time (months)	TP [a]	Hydroxycinnamates (mg chlorogenic/100g)	Epicatechin (mg epicatechin/100g)	Flavonol glycosides (mg rutin/100g)	ORAC [a]	FRAP [a]
Fresh			194	74.41 ± 1.57a	13.44 ± 2.77a	4.72 ± 0.17b	1312	1432
Freezing	-23	3	145	27.90 ± 2.63c	11.71 ± 3.69a	5.32 ± 0.49b	1257	995
Freezing	-23	6	96	17.11 ± 2.20d	nd	5.20 ± 0.55b	941	598
Freezing	-70	3	210	70.61 ± 1.03b	10.82 ± 1.36a	4.87 ± 0.31b	2460	1670
Freezing	-70	6	200	76.82 ± 3.73a	13.13 ± 1.41a	6.04 ± 0.65a	2378	2085

[a] Total phenolic contents were reported as mg gallic acid/100g fw while ORAC and FRAP were reported as μmoles Trolox equivalent/100g fw (*12*). Each value in table is means ± standard deviation (n=2). The values with different letters in each column indicate a significant difference at $\alpha = 0.05$. Tempt is temperature.

The anthocyanin pigment profiles appear to be very similar; however, statistical analysis indicates significant changes for some of the individual pigments. There was some evidence that cyanidin glycosides were more labile than pelargonidin and peonidin glycosides since the latter showed significant percentage increases at –23 °C storage. Partial hydrolysis of cyanidin-3-rutinoside producing cyanidin-3-glucoside might account for its significant decrease at –23 °C. Forni et al. (*18*) reported increases of cyanidin-3-glucoside from cyanidin-3-rutinoside partial hydrolysis in sweet cherries during osmodehydration and storage. Francis (*19*) reported that both cyanidin-3-glucoside is formed during acid hydrolysis of cyanidin-3-rutinoside.

Formation of minor unidentified anthocyanin peaks was detected in cherries stored at –23 °C after 3 months and the amounts increased at 6 months. Possible anthocyanin degradation products are anthocyanin-polyphenolic condensation compounds (*16, 17*).

Total phenolics showed marked decreases at –23 °C storage and apparent increases at –70 °C (Table 3.4). Hydroxycinnamates and epicatechin showed dramatic losses at –23 °C while flavonol glycosides were relatively unchanged. Native polyphenoloxidase very likely plays a major role in polyphenolic degradation at –23 °C (*37*). Several investigators have shown that chlorogenic acid and epicatechin are favored substrates for polyphenoloxidase (*2, 30, 43*). Other investigators have demonstrated that flavonol glycosides are affected by only a small degree by polyphenoloxidase (*2, 50*). Robards et al. (*41*) reviewed that the

quercetin and quercetin-3-galactoside are not oxidized by polyphenoloxidase because of the unsaturated bond in the C-ring. Kader et al. (*29*) reported that anthocyanins with ortho phenolic substitution in the B-ring such as cyanidin glycosides can degraded via a coupled oxidation with the quinone of chlorogenic acid, which have been formed from polyphenoloxidase. Our results suggested that the pronounced degradation of polyphenolics and anthocyanins that occurred during storage at –23 °C might be due to native enzymes. This degradative loss was minimal at –70 °C storage.

Changes in anthocyanins and polyphenolics during canning and canned storage

Table 3.5 shows the effect of canning and storage at 2 and 22 °C on the anthocyanin pigments of Bing cherries. While 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. Approximately half of the anthocyanins were leached into the syrup with canning. The anthocyanin pigment profiles of fresh cherries, canned cherries and syrup were similar, however there were some apparent differences. The proportions of cyanidin and pelargonidin rutinosides in cherries slightly decreased with canning while cyanidin and pelargonidin glucosides increased. Partial hydrolysis of rutinose to glucose can account for these changes (*19, 60*). Substantial anthocyanin

degradation occurred after 5 months storage at 2 °C (12%) and 22 °C (42%). Weinert et al. (*59*) showed similar anthocyanin losses for canned plums stored for 47 days 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 3.6 shows how Bing polyphenolics are affected by canning and storage at 2 and 22 °C for 5 months 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/100g) and in syrup (0.4-0.5 mg/100g) 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 months storage at both 2 and 22 °C. The degradation of epicatechin increased significantly at 22°C. Flavonol glycosides were more stable showing a significant decrease at 2 °C and an apparent increase at 22 °C. There was evidence for an approximate two time increase of caffeic acid in cherries and syrup after 5 month storage at 22 °C (data not shown).

Table 3.5: Relative anthocyanin composition of fresh and canned cherries and their syrup stored at 0 and 5 months at two different storage temperatures (2 °C and 22°C)

Samples	Portion	Tempt (°C)	Time (months)	ACN (mg/100g) [a]	Anthocyanins (% total peak area at 520 mn)				
					Cyanidin-3-glucoside	Cyanidin-3-rutinoside	Pelargonidin-3-glucoside	Pelargonidin-3-rutinoside	Peonidin-3-rutinoside
Fresh Cherries				63.7	8.05 ± 0.08	85.21 ± 0.17	0.56 ± 0.03	0.91 ± 0.02	5.26 ± 0.09
Canned cherries		2	0	64.4					
	Cherries			35.1	9.13 ± 0.16	84.47 ± 0.21	0.61 ± 0.01	0.80 ± 0.01	4.98 ± 0.10
	Syrup			29.9	7.50 ± 0.18	85.48 ± 0.36	0.66 ± 0.07	0.90 ± 0.05	5.46 ± 0.36
Canned cherries		2	5	57.0					
	Cherries			28.0	9.02 ± 0.20	84.08 ± 0.09	0.79 ± 0.04	0.91 ± 0.06	5.20 ± 0.09
	Syrup			29.0	7.15 ± 0.06	85.94 ± 0.14	0.83 ± 0.08	0.91 ± 0.05	5.17 ± 0.09
Canned cherries	Total	22	0	68.9					
	Cherries			33.3	8.68 ± 0.51	84.61 ± 0.29	0.71 ± 0.07	0.82 ± 0.07	5.17 ± 0.32
	Syrup			35.6	8.00 ± 0.33	85.05 ± 0.40	0.75 ± 0.02	0.84 ± 0.15	5.36 ± 0.55
Canned cherries	Total	22	5	39.7					
	Cherries			19.7	9.05 ± 0.19	83.85 ± 0.19	1.20 ± 0.06	0.93 ± 0.05	4.97 ± 0.07
	Syrup			20.0	8.38 ± 0.10	84.53 ± 0.21	1.20 ± 0.03	0.99 ± 0.09	4.90 ± 0.17

[a] Anthocyanin content were reported as mg cyanidin-3-glucoside/100g fw (*12*). Each value in table is mean ± standard deviation (n=2). Tempt is temperature.

Table 3.6: 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 month storage

Samples	Portion	Tempt (°C)	Time (months)	TP [b]	Hydroxycinnamates (mg chlorogenic /100g)	Epicatechin (mg epicatechin /100g)	Flavonol glycosides (mg rutin /100g)	ORAC [b]	FRAP [b]
Fresh Cherries				194	70.31 ± 3.42B	11.30 ± 0.82A	6.41 ± 0.09B	1312	1432
Canned cherries		2	0	259	87.70 ± 0.81Aa	10.00 ± 1.14Aay	8.62 ± 0.19Aa	1742	1997
	Cherries	2	0	117	42.86 ± 0.55	5.58 ± 0.90	4.40 ± 0.18	892	959
	Syrup	2	0	141	44.84 ± 0.51	4.43 ± 0.23	4.22 ± 0.29	851	1038
Canned cherries		2	5	235	80.36 ± 1.21b	7.99 ± 0.33ax	7.29 ± 0.51b	2957	2059
	Cherries	2	5	127	40.96 ± 1.74	4.29 ± 0.28	3.93 ± 0.28	1418	1007
	Syrup	2	5	108	39.40 ± 1.21	3.70 ± 0.09	3.37 ± 0.25	1538	1052
Canned cherries		22	0	233	86.11 ± 3.19Aa	8.58 ± 0.29Bby	6.95 ± 0.71Bb	1845	1855
	Cherries	22	0	113	41.93 ± 2.18	4.16 ± 0.35	4.36 ± 0.41	898	879
	Syrup	22	0	120	44.17 ± 1.14	4.43 ± 0.16	2.59 ± 0.03	947	975
Canned cherries		22	5	231	60.50 ± 1.37c	7.22 ± 0.30bx	7.60 ± 0.03b	2896	1903
	Cherries	22	5	121	39.40 ± 0.64	3.54 ± 0.25	3.96 ± 0.15	1276	898
	Syrup	22	5	110	21.10 ± 0.73	3.69 ± 0.08	3.64 ± 0.20	1620	1002

[a] Total phenolic content were reported as mg gallic acid/100g fw while ORAC and FRAP were reported as μmoles Trolox equivalent/100g fw (*12*). Each value in table is mean ± standard deviation (n=2). The values with different letters in each column indicate a significant difference at $\alpha = 0.05$. Tempt is temperature.

Changes in anthocyanins and polyphenolics during brining

The anthocyanin composition of fresh Bing and Royal Ann cherries are compared with the brined fruit and spent brine after one year storage at 22 °C in Table 3.7. The anthocyanin pigments become colorless after reacting with SO_2 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 contains 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 cherry 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 (*19*) reported that cyanidin-3-glucoside is 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.

Table 3.7: Relative anthocyanin composition of fresh cherries, brined cherries, and their spent brine solution

Cultivar		ACN[a] (mg/100g)	Anthocyanins (% total peak area at 520 mn)						
			Unknown	Cyanidin-3-glucoside	Cyanidin-3-rutinoside	Pelargonidin-3-glucoside	Pelargonidin-3-rutinoside	Peonidin-3-glucoside	Peonidin-3-rutinoside
Bing	Fresh	26.1	nd	8.05 ± 0.08	85.20 ± 0.18	0.56 ± 0.03	0.92 ± 0.02	nd	8.11 ± 0.17
	Brined cherries	0.5	nd	6.82 ± 0.44*	88.28 ± 0.22*	nd*	nd*	nd	4.89 ± 0.27*
	Spent Brine solution	11.1	1.87 ± 0.06*	7.22 ± 0.08*	84.8 ± 0.12*	nd*	0.90 ± 0.01	0.56 ± 0.01*	4.64 ± 0.08*
Royal Ann	Fresh	0.63	nd	nd	88.10 ± 1.45	11.90 ± 1.45	nd	nd	nd
	Brined cherries	0.1	nd	nd	nd*	nd*	nd	nd	nd
	Spent Brine solution	0.1	nd	27.44 ± 0.27*	72.56 ± 0.27*	nd*	nd	nd	nd

[a] Anthocyanin content (ACN) were reported as mg cyanidin-3-glucoside/100g fw (*12*). Each value in table is mean ± standard deviation (n=2). A t- test was used to compare fresh cherries with brined cherries or spent brine solution. * means a significant difference from fresh cherries at α=0.05. Each cultivar was analyzed separately.

The HPLC polyphenolic profiles for fresh Bing cherries are compared with the spent brine in Figure 3.9, and a similar comparison for Royal Ann is shown in Figure 3.10. No polyphenolics was detected in the brined fruits. In addition to leaching into the brine during storage, there would be losses during the extensive water washing to remove residual SO_2. Most of identified hydroxycinnamates or flavonol glycosides were 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 was identified. Their UV-Visible spectra were similar to those of epicatechin and procyanidins, which have no unstauration in the C-Ring.

Figure 3.9: HPLC polyphenolic profile of Bing cherries (A) and Bing spent brine solution (B) at 260 nm, 280 nm, 320 nm, 370 nm, and 520 nm. 1: neochlorogenic acid, 2: 3'-*p*-coumaroylquinic acid, 3: chlorogenic acid, 4: epicatechin, 5: quercetin-3-rutinoside, 6: quercetin-3-glucoside, 7: kaempferol-3-rutinoside, HC: unidentified hydroxycinnamates, UP: unidentified polyphenolics, A: anthocyanin

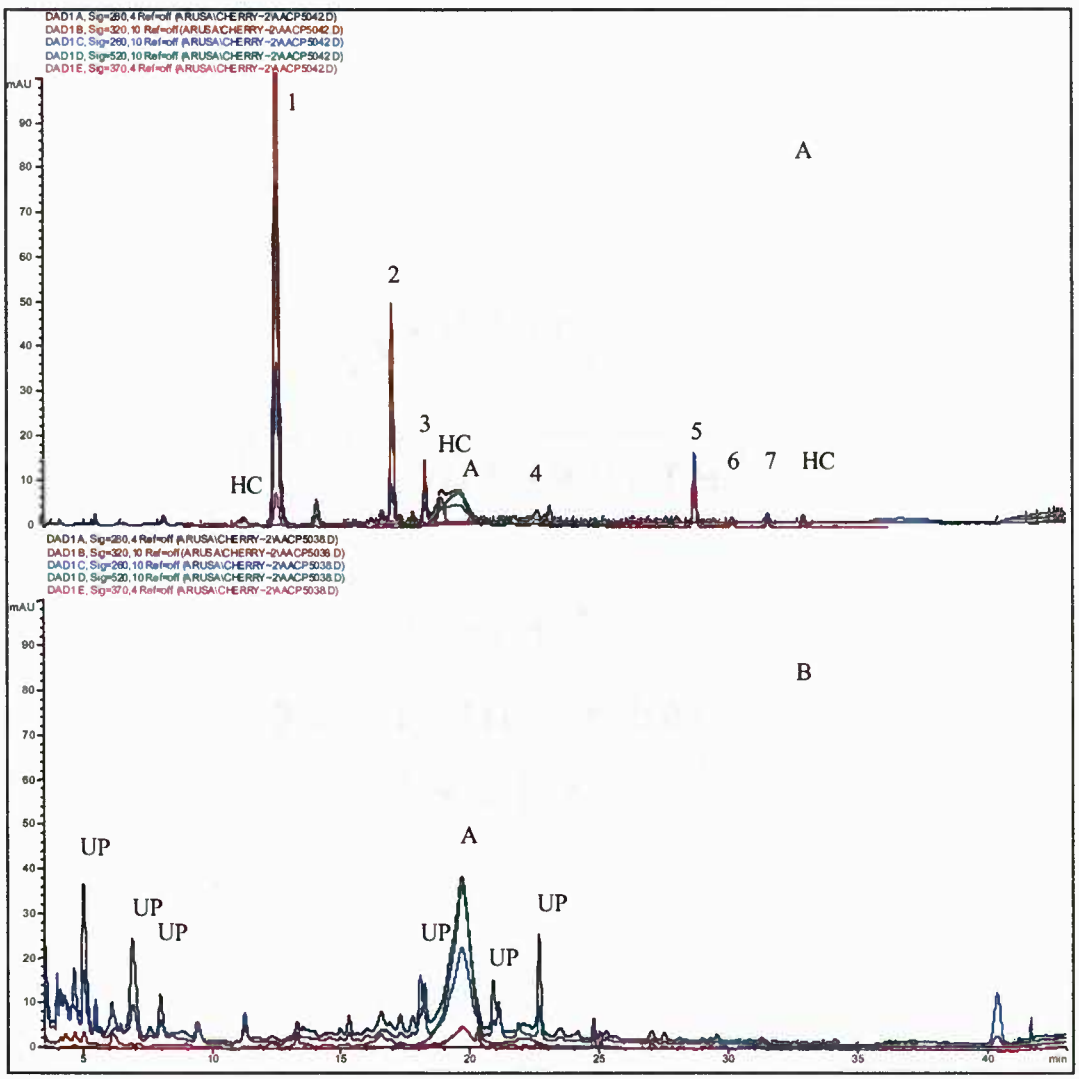
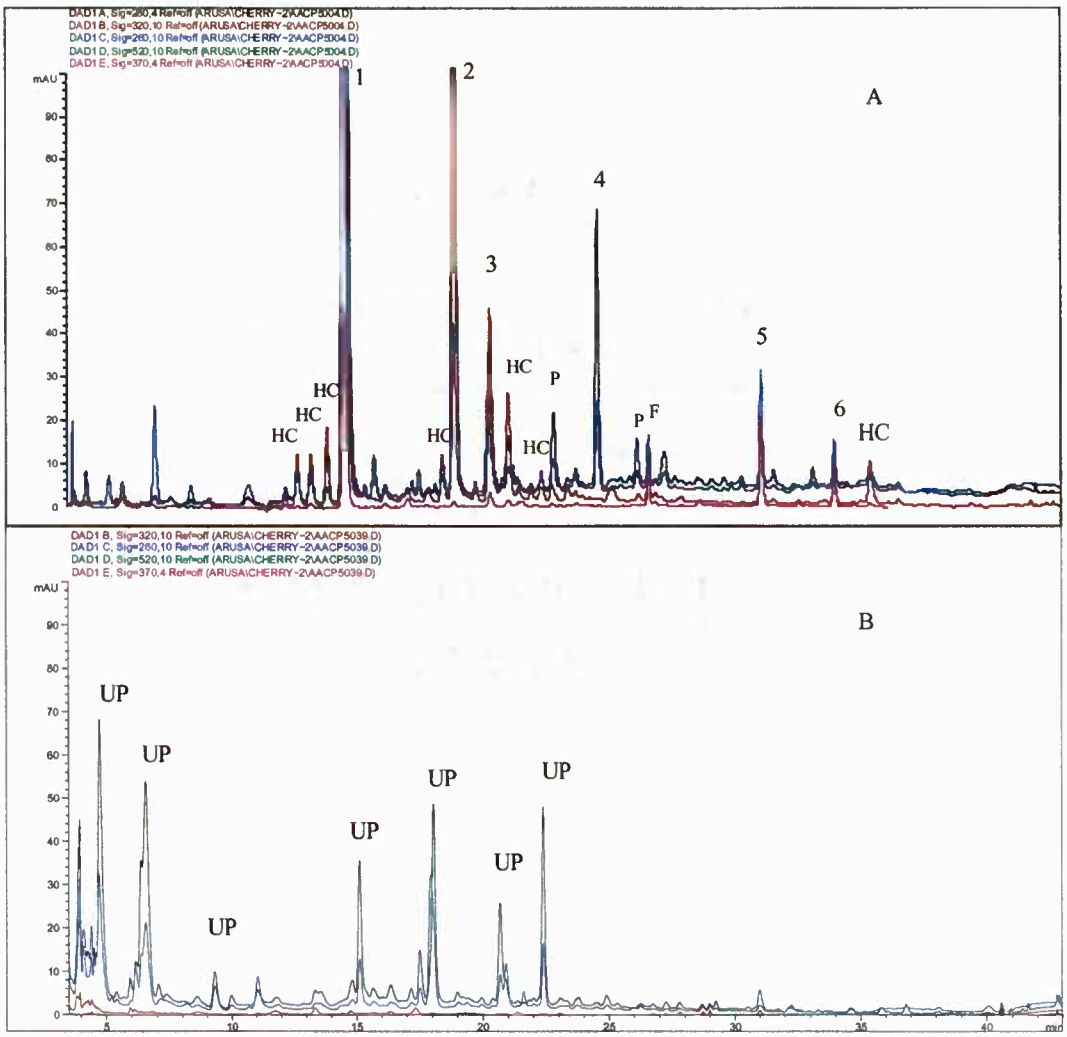


Figure 3.10: HPLC polyphenolics profile of Royal Ann cherries (A) and Royal Ann spent brine solution (B) at 260 nm, 280 nm, 320 nm, 370 nm, and 520 nm. 1: neochlorogenic acid, 2: 3'-*p*-coumaroylquinic acid, 3: chlorogenic acid, 4: epicatechin, 5: quercetin-3-rutinoside, 6: kaempferol-3-rutinoside, HC: unidentified hydroxycinnamates, P: procyanidins, UP: unidentified polyphenolics, F: unidentified flavonols



Relevance of anthocyanin and polyphenolic composition to antioxidant properties

In an earlier publication (*12*), 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. Tables 3.2, 3.4, and 3.6 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 since 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 3.2). While Bing skins had the highest anthocyanin content, their total phenolic 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 as follows: 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).

Robards et al. (*41*) reviewed the ORAC activity of polyphenolic compounds as follows: quercetin>kaempferol>epicatechin>caffeic acid>*p*-coumaric acid>pelargonidin>quercetin-3-rutinoside. Kähkönen and Heinonen (*31*) reported that the antioxidant power of anthocyanins depended on the type of anthocyanidin and the glycosylation pattern. Wang et al. (*53*) reported the ORAC activity of anthocyanin as decrease 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 (*38*). It has been recognized that synergistic effects between polyphenolics and other phytochemicals can also enhance antioxidant activity (*32, 44*).

Total phenolics declined during frozen storage of Bing cherries at –23 °C, and both ORAC and FRAP also decreased (Table 3.4). 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 since the structure is associated with the antioxidant activity. While canning resulted in increased levels of total phenolics, both ORAC and FRAP increased by even greater orders of magnitude (Table 3.6). ORAC in particular, but FRAP as well 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 (*3, 4, 35*).

CONCLUSIONS

While 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 three 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. While Bing cherries were highest in anthocyanins and the only cultivar with pigmentation in the flesh, it was the only cultivar where 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. Since 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 due to native enzymes, particularly polyphenoloxidase. During canning, approximately half of the anthocyanins and polyphenolics were

redistributed to the syrup 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.

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CHAPTER V. SUMMARY

The weight distribution of cherries was found to be skins (13-18 %), flesh (60-71 %), and pits (5-8%). Anthocyanins and polyphenolics predominated in skins, providing high antioxidant activities. Bing cherries had anthocyanin pigmentation in skins, flesh, and pits, while the red blushed Rainier, Royal Ann, and the brilliant red Montmorency cherries had pigmentation limited to mainly skins. The distribution of polyphenolics was variable. Hydroxycinnamates were widely distributed throughout skins, flesh, and pits; flavonol glycosides predominated in skins; and flavanols were prevalent in pits. The major anthocyanin in sweet cherries was cyanidin-3-rutinoside while in sour cherries it was cyanidin-3-glucosylrutinoside. The major phenolics in sweet and sour cherries were identified and measured as hydroxycinnamates, flavonol glycosides, and flavanols. About 70-80% of the polyphenolics in sweet cherries were hydroxycinnamates such as neochlorogenic acid, chlorogenic acid, and 3'-*p*-coumaroylquinic acid while about 50% of the polyphenolics in sour cherries were flavanols.

Processing and storage had pronounced effect on anthocyanins, phenolics, and antioxidant activities. Over 75% of anthocyanins were destroyed after 6 month storage at –23 °C while they were relatively stable at -70° C storage. The degradation of anthocyanins during frozen storage is possibly related to the native enzyme, polyphenoloxidase. Approximately 50% of anthocyanins and

polyphenolics of cherries were redistributed to the syrup during canning. However, there was little loss of total anthocyanins and a marked increase of total antioxidant activity after canning and during storage. The antioxidant activities from Maillard browning products are the possible explanation. Anthocyanin-rutinosides underwent partial hydrolysis during frozen storage and canning. Hydrolysis of hydroxycinnamates to liberate caffeic acid occurred with canning. During brining, roughly half of the polyphenolics and anthocyanins were leached into the brine solution. Washing of the brined cherries to reduce SO_2 levels resulted in removal of essentially all the anthocyanins and polyphenolics from the brined cherries. Cherry anthocyanins could be recovered from spent brine. Polyphenolics of cherry and spent brine was qualitatively different. Hydroxycinnamates and flavonol glycosides disappeared with the formation of unidentified polyphenolic derivatives, which possessed antioxidant activities.

Cherry processing waste such as skins, pits, and spent brine solution contained substantial amounts of anthocyanins and polyphenolics. They could be a potential source of natural colorants, nutraceuticals and natural antioxidants.

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