Total Anthocyanins and Total Phenolics of Fresh and Processed Cherries and Their Antioxidant Properties

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ABSTRACT: Total anthocyanins, total phenolics, and the antioxidant activities of 1 sour cherry cultivar (*Prunus cerasus* L.) and 3 sweet cherry cultivars (*P. avium* L.) were determined. Bing cherries were highest in anthocyanins, whereas Montmorency cherries were highest in total phenolics and antioxidant activities (oxygen radical absorbance capacity and ferric reducing antioxidant power). Total phenolics and anthocyanins for all cultivars were concentrated in the skin. More than 75% of anthocyanins in frozen Bing cherries were destroyed after 6 mo of storage at –23 °C. During canning, about half the anthocyanins and polyphenolics leached from the fruits into the syrup with little total loss. Spent cherry brine contained substantial anthocyanins and polyphenolics.

Keywords: cherries, canning, brining, frozen storage, antioxidant properties

Introduction

Epidemiological studies show a strong association between fruit and vegetable consumption and reduced risk of several degenerative diseases such as cancer, cardiovascular disease, and stroke, which are caused by oxidative stress (Ames and others 1993; Ma and Kinner 2002). Specifically, cherry consumption has been reported to alleviate arthritis and gout-related pain (Wang and others 1999b; Seeram and others 2002). The beneficial effects from fruits and vegetables have been ascribed to natural antioxidants such as anthocyanins and polyphenolics (Robards and others 1999; Kaur and Kapoor 2001). Sweet cherries (*Prunus avium* L.) and sour cherries (*Prunus cerasus* L.) contain substantial amounts of anthocyanins and polyphenolics (Gao and Mazza 1995; Wang and others 1997; Friedrich and Lee 1998; Wang and others 1999a). Comparative data on sweet and sour cherry composition using the same analytical methodologies, however, are limited.

Anthocyanins and polyphenolics are not uniformly distributed in fruit tissue. Skin contains high amounts of polyphenolics and anthocyanins (Tomás-Barberán and others 2001). 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, whereas in Rainier, Royal Ann, and Montmorency cherries they are limited to the skins. Royal Ann and Rainier cherries are yellow with a red blush, whereas Montmorency cherries are intensely red in skin color. The flesh of all 3 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 could juice-processing wastes, which include skins as well as pits.

Several methods have been used to determine the antioxidant activity of fruits (Meyer and others 1997; Heinonen and others 1998; Kähkönen and others 2001; Seeram and others 2002). Heinonen and others (1998) compared the in vitro antioxidant activity of several fruits. Sweet cherries have higher antioxidant activity than blueberries and strawberries and are ranked as the 1st and the 3rd in their antioxidant activities in liposome and low-density lipoprotein, respectively. Seeram and others (2002) reported that the anti-inflammatory activity of sweet cherries was higher than that of 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 (Wang and others 1996; Kalt and others 1999, 2000, 2001; Moyer and others 2002). The 2 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 (Ou and others 2002).

In the United States, the cherry season is short, lasting from mid-May to mid-August across the entire country, and only for a few weeks for any specific region. In 1999 to 2001, about 40% of the sweet cherry production was processed as brined (70%), canned (12%), and frozen, dried, or used for juice (18%) (ERS 2002). Approximately 99% of tart cherry crop was processed as frozen (>50%), canned (>33%), and brined, dried, or used for juice (>10%) (ERS 2002). The primary use of processed sour cherries is in baking and cooking. Although there have been several investigations on the effect of processing on anthocyanins and polyphenolics in fruits other than cherries (Kalt and others 2000; Skrede and others 2000), 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 (Do and others 1976; Polesello and Bonzini 1977; Forni and others 1993). Because of their possible health benefits, there is intense interest in the

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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 to measure their distribution in fruits. Evaluation of the effects 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 Oregon State Univ. (OSU) Lewis Brown horticultural farm (Corvallis, Ore., U.S.A.) from late-June to mid-July 2001. Fresh Rainier cherries were obtained from the Mid-Columbia Experiment Station (Hood River, Ore., U.S.A.) in mid-July 2001. Oregon Cherry Growers, Inc. (Salem, Ore., U.S.A.) provided Bing and Royal Ann cherries for the brining processing experiment in June 2000 and also provided Bing cherries for canning and frozen storage experiments in June 2001. Upon receipt at the Dept. of Food Science and Technology, OSU, Corvallis, Ore., the cherries were stored at 2 °C before subsequent sample preparation or processing.

Sample preparation of fresh cherries

Cherry samples (about 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 (about 200 g) were also pitted using a household hand cherry pitter for studies on edible portions of cherries. Samples were prepared by 2 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 (about 10 g) was mixed with 20 mL acetone, sonicated with an ultrasonic cleaning device (Branson Cleaning Equipment Corp., Shelton, Conn., U.S.A.) for 10 min, and then filtered using Whatman nr 1 paper (Whatman Inc., Clifton, N.J., U.S.A.) on a Büchner funnel. The filter cake was re-extracted with 10 mL 70% acetone (30% water and 70% acetone, vol/vol) twice. Filtrates were combined and mixed with 80 mL chloroform and then centrifuged at 170 × *g* for 20 min with an IEC Intl. centrifuge (Model UV, International Equipment Co., Boston, Mass., U.S.A.). The upper aqueous phase was collected and evaporated under reduced pressure at 40 °C until the residual acetone was removed (about 15 min). The aqueous extract was made up to 25 mL with acidified water (0.01% HCl [vol/vol] in deionized, distilled water) and stored at –70 °C until subsequent analyses. Sample extractions were replicated twice.

Cherry processing

Frozen cherries. Stemmed Bing cherries (about 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 (Nalge Nunc Intl., Rochester, N.Y., U.S.A.) 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 mo of storage.

Canned cherries. Stemmed Bing cherries (2.5 kg) were washed and pitted using a household hand cherry pitter. Nr 303 cans with dark fruit enamel were filled with pitted cherries and 19 °Brix sucrose syrup (C&H Superfine sugar diluted to 19 °Brix with water; C&H Sugar Co., Crockett, Calif., U.S.A.) at 80 °C was added. The cans were exhausted on a steam bath for 2 min and 30 s and sealed with nr 303 lids on a Canco 006 Steam-flow can seamer (Pegasus Food Machinery Corp., Carson City, Nev., U.S.A.). 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 for 5 mo.

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 sodium metabisulfite, 202 g calcium chloride, and 10 g citric acid in 10 L of water. This gave a solution that was 2.2% sodium metabisulfite, 2% calcium chloride, and 0.1% 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 mo at 22 °C. The spent brine was recovered for analysis, and the cherries were washed with running cold water for about 5 d 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 and others (1970), 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 on juice expressed from fresh fruits using an Auto Abbe refractometer 10500 (Reichert-Jung, Leica Inc., N.Y., U.S.A.). The instrument was set to measure % total soluble solids using the temperature-compensated mode. A Brinkmann 605 pH meter (Brinkmann, Methrohm City, Switzerland) was used for pH determination.

Anthocyanin pigment content and polymeric color indices

The monomeric anthocyanin pigment content of the aqueous extracts was determined using the pH-differential method (Giusti and Wrolstad 2001). A Shimadzu 300-UV spectrophotometer (Shimadzu Inc., Kyoto, Japan) and a 1-cm path length disposable cell were used for spectral measurements at 510 and 700 nm. Pigment content was calculated as milligrams cyanidin-3-glucoside/100 g fresh weight using an extinction coefficient of 26900 L/cm/mol and molecular weight of 449.2 g/mol.

Color density, polymeric color, and percent polymeric color were determined using the bisulfite bleaching method as described by Giusti and Wrolstad (2001).

Total phenolics

Total phenolic content was determined using the modified Folin-Ciocalteu procedure described by Singleton and Rossi (1965). A 0.5-mL sample of the aqueous extract or a series of gallic acid standards (0, 40, 80, 120, 160, and 200 ppm) were mixed with 0.5 mL of the Folin-Ciocalteu reagent (Sigma Chemical Co., St. Louis, Mo., U.S.A.) and 7.5 mL 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 40 °C water bath for 20 min and then immediately cooled in an ice bath before measuring the absorbance at 755 nm. Results were expressed as milligrams of gallic acid equivalent per gram of fresh weight.

Antioxidant activities

Antioxidant activities were determined by ORAC and FRAP assays at the Linus Pauling Inst., Oregon State Univ. The ORAC assay was performed as described by Cao and others (1993) and adapted for use in a 96-well microplate fluorometer (model Cytofluor 4000;

Table 1—°Brix and pH of 4 cherry cultivars[a]

Cultivar	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

[a]Values are mean ± standard deviations, $n = 3$

PerSeptive Biosystems, Framingham, Mass., U.S.A.). ORAC values, derived from triplicate analyses, are expressed as μmoles Trolox equivalent (TE) per gram of fresh weight. Trolox is a water-soluble tocopherol analog used as a reference compound for antioxidant activity. The FRAP assay (Benzie and Strain 1996) was adapted for use in a 96-well microplate spectrophotometer (ThermoMax, Molecular Devices, Foster City, Calif., U.S.A.). FRAP values, derived from triplicate analyses, are expressed as μmoles TE per gram of fresh weight.

Statistical analysis

All processing experiments and analytical determinations were replicated twice. The distribution of anthocyanins, total phenolics, and antioxidant activities in 4 cherry cultivars and the effect of processing on cherry anthocyanins, total phenolics, and antioxidant activities was ascertained by analysis of variance using S-Plus 4.5 (MathSoft, Seattle, Wash., U.S.A.). To assess the effect of frozen storage on total anthocyanins, total phenolics, and antioxidant activities, a 1-factor ANOVA model was used, the factor being the combination of frozen temperature and storage time. The effects 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 1-factor ANOVA model was used with the levels of the factors as follows: fresh cherries, canned cherries at 2 storage temperatures. To evaluate the effect of storage after canning, a 2-way ANOVA was used with time and temperature as factors. To assess the effect of brining, a 2-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 ($P = 0.05$). The Pearson correlation matrix was used to determine the correlation among anthocyanins, total phenolics, and antioxidant activities. A 95% significance 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 1. The proportions of skins, flesh, and pits and the physical properties are presented in Table 2. The proportions of the 4 cultivars were similar with flesh ranging from 60.4% to 71.4%, skins from 13.5% to 18.1%, and pits from 5.5% to 7.9%. Inherent losses (9.6% to 14.7%) occurred in peeling, pitting, and weighing the samples.

Distribution of anthocyanin pigments

Table 2 presents the anthocyanin content of the 4 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, whereas others had pigmentation in skins and pits. The highest anthocyanin concentration (60.6 mg/100 g) was found in Bing skins. The brilliant red skins of Montmorency cherries had the 2nd highest anthocyanin concentration (36.5 mg/100 g). Royal Ann and Rainier cherries are yellow-colored with a red blush and had light anthocyanin pigmentation, 2.2 mg/100 g and 2.1 mg/100 g, respectively.

Anthocyanin content of the edible portion (skin plus flesh) was highest for Bing cherries (29.7 mg/100 g), followed by Montmorency (8.7 mg/100 g), Rainier (0.5 mg/100 g), and Royal Ann cherries (0.5 mg/100 g). 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/100 g. These

Table 2—Total anthocyanins, total phenolics, and antioxidant properties of edible portion, flesh, pits, and skins of 4 cherry cultivars[a,b]

Cultivar	Portion	Distribution (%)	Anthocyanins (mg cyn-3-glu /100 g fw)	Total phenolics (mg GAE/g fw)	ORAC (μmoles TE/g fw)	FRAP (μmoles TE/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				

[a]FRAP = ferric reducing antioxidant power; fw = fresh weight; GAE = gallic acid equivalent; ORAC = oxygen radical absorbance capacity; TE = Trolox equivalent
[b]Values are mean ± standard deviations, $n = 3$ for percent distribution while $n = 2$ for anthocyanins, total phenolics, ORAC, and FRAP

Table 3—Effect of frozen storage temperature and time on total anthocyanins, percent polymeric color, total phenolics, ORAC, and FRAP of pitted Bing cherries[a,b]

Storage temperature (°C)	Storage time (mo)	Anthocyanins (mg cyn-3-glu/100 g fw)	Polymeric color (%)	Total phenolics (mg GAE/g fw)	ORAC (μmoles TE/g fw)	FRAP (μmoles TE/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

[a]FRAP = ferric reducing antioxidant power; fw = fresh weight; GAE = gallic acid equivalent; ORAC = oxygen radical absorbance capacity; TE = Trolox equivalent
[b]Values are mean ± standard deviations, $n = 2$. Different letters within the same column indicate significant difference at $P \leq 0.05$

values for Bing cherries are much lower than the value (224.7 mg/100 g) reported by Gao and Mazza (1995), but were comparable to the quantity (25.95 mg/100 g) reported by Seeram and others (2002). The amounts of Montmorency anthocyanins in our study were lower than the values (12.5 mg/100 g to 25.0 mg/100 g) reported by Wang and others (1997). The total anthocyanin content for both Rainier and Royal Ann cherries was lower than the amounts for "light-colored" cherries (2 mg/100 g to 41 mg/100 g) reported by Gao and Mazza (1995).

Several factors can influence anthocyanin content in cherries, such as cultivar, maturity, geographic location, and environmental factors such as light, temperature, and various stresses (Kalt and others 1999; Tomás-Barberán and Espín 2001).

Distribution of total phenolics

Table 2 shows the distribution of total phenolics in skins, flesh, and pits for the 4 cherry cultivars. Total phenolics were highest in skins, intermediate in flesh, and lowest in pits. Montmorency cultivar had the highest amounts in all 3 portions. Although 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 (0.75 mg/g). Mozetic and others (2002) reported the total phenolics of Bing and Royal Ann to be 0.97 mg/g and 1.44 mg/g, respectively. Heinonen and others (1998) reported the total phenolics of Bing cherries to be 0.80 mg/g with hydroxycinnamates being the major polyphenolic class of compounds. Our values for total phenolics are considerably higher than those of both studies.

Antioxidant activities of cherry components

Cherry skins were highest in antioxidant activities whereas pits were lowest (Table 2). Results of ORAC and FRAP followed the same trends: the 2 measurements having a positive correlation of $r = 0.98$. Montmorency skins were the highest in ORAC and FRAP, followed by Bing, Royal Ann, and Rainier skins, respectively (Table 2). Obviously, anthocyanin content *e* by itself 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 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 (Wang and others 1996; Meyer and others 1997; Kalt and others 1999; Moyer and others 2002).

Effect of frozen storage

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

Anthocyanin degradation in frozen cherries is very likely related to the presence of native enzymes, particularly polyphenoloxidase, which has been shown to be very active in both cherry flesh and skins (Pifferi and Cultrera 1974). Polyphenoloxidase can accelerate anthocyanin degradation in the presence of polyphenolics, particularly chlorogenic acid, which is one of the major phenolic compounds in cherries (Pifferi and Cultrera 1974). Polyphenoloxidase activity is temperature-dependent (Manzocco and others 1998). Kader and others (1998) showed that in blueberries, polyphenoloxidase oxidized chlorogenic acid to a quinine, which would 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 mo and 50% after 6 mo, although not as extremely as anthocyanins. A decrease in antioxidant activities (28% for ORAC and 58% for FRAP) during 6 mo of storage at –23 °C was found, whereas there was a surprising apparent increase in antioxidant activities 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.

Effect of canning

Changes of anthocyanin content, total phenolics, and antioxidant activities of pitted Bing cherries canned in light syrup during canning and storage are shown in Table 4. 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

Table 4—Effect of canning, storage temperature, and storage time on total anthocyanins, percent polymeric color, total phenolics, ORAC, and FRAP of pitted Bing cherries and canned Bing cherries in light syrup[a,b]

Sample	Storage temperature (°C)	Storage time (m)	Anthocyanins (mg cyn-3-glu/100 g fw)	Polymeric color (%)	Total phenolics (mg GAE/g fw)	ORAC (μmoles TE/g fw)	FRAP (μmoles TE/g fw)
Fresh		0	63.7 ± 0.9a	12.5 ± 2.3	1.94 ± 0.17b	13.12 ± 1.22b	14.32 ± 0.39b
Total[c]	2	0	64.4 ± 1.3aa		2.59 ± 0.12a	17.42 ± 2.91b	19.97 ± 0.53a
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[c]	2	5	57.0 ± 5.4a		2.35 ± 0.07a	29.57 ± 1.42a	20.59 ± 0.92a
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[c]	22	0	68.9 ± 6.9Aa		2.33 ± 0.23Aa	18.45 ± 1.28b	18.55 ± 2.14b
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[c]	22	5	39.7 ± 1.2b		2.31 ± 0.06a	28.96 ± 0.89a	19.03 ± 0.43b
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

[a]FRAP = ferric reducing antioxidant power; fw = fresh weight; GAE = gallic acid equivalent; ORAC = oxygen radical absorbance capacity; TE = Trolox equivalent
[b]Values are mean ± standard deviations, $n = 2$. Different letters within the same column indicate significant difference at $P \leq 0.05$.
[c]Cherry and syrup values combined

Table 5—Effect of brining on total anthocyanins, total phenolics, and antioxidant activities of pitted Bing and Royal Ann cherries and their brine solution[a,b]

Cultivar	Sample	Processing	Anthocyanins (mg cyn-3-glu/100 g fw)	Total phenolics (mg GAE/g fw)	ORAC (μmoles TE/g fw)	FRAP (μmoles TE/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
	Brine 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[c]	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
	Brine 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[c]	0.1 ± 0.0	1.18 ± 0.04	13.6 ± 0.37	10.4 ± 0.19

[a]FRAP = ferric reducing antioxidant power; fw = fresh weight; GAE = gallic acid equivalent; ORAC = oxygen radical absorbance capacity; TE = Trolox equivalent
[b]Values are mean ± standard deviations, $n = 2$. Different letters within the same column indicate significant difference at $P \leq 0.05$.
[c]Due to the interference of brine solution with the determination of total phenolics, ORAC, and FRAP, values are subtracted by fresh brined solution (blank).

temperature may increase membrane permeability in the macerated peel tissue facilitating phenolic extraction (Spanos and others 1990). Moreover, with the breakdown of the cellular constituents, bound phenolic compounds may be released (Dewanto and others 2002). Kalt and others (2000) reported that extraction of blueberries at 60 °C resulted in higher total phenolics and antioxidant activities. Anese and others (1999) reported that heating increased the antioxidant potential in tomato juice as a consequence of the formation of Maillard reaction products. The increase of percent 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 mo of storage at 22 °C, a significant ($P \leq 0.05$) decrease in total anthocyanins was observed, although there was no significant decrease in total phenolics at either 2 °C 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, whereas ORAC values increased after 5 mo of storage at either 2 °C 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 (Eichner 1981; Lingnert and Eriksson 1981; Anese and others 1999; Anthony and others 2000; Morales and Babbel 2002).

Effect of brining

Changes of total anthocyanins, total phenolics, ORAC, and FRAP in Bing and Royal Ann cherries after brining are presented in Table 5. Little of the 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, glacéed cherries, or other food products.

Approximately 50% of the anthocyanins and total phenolics were leached from the cherries into the brine solution. Anthocyanin losses of Bing and Royal Ann cherry fruits were 57.5% and 84.1%, respectively, whereas losses of total phenolics were 91.1% and 50.8%. The spent brine is rich in antioxidant activities, presumably because of the anthocyanins and polyphenolics. With respect to the 2 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, whereas 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.

Conclusions

In summary, 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. About 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. In addition to cherries themselves, cherry-processing wastes may be potential sources for natural colorants, nutraceuticals, and natural antioxidants.

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