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Processing and Storage Effects on Monomeric Anthocyanins, Percent Polymeric Color, and Antioxidant Capacity of Processed Black Raspberry Products

A. HAGER, L.R. HOWARD, R.L. PRIOR, AND C. BROWNMILLER

ABSTRACT: This study evaluated the effects of processing and 6 mo of storage on total monomeric anthocyanins, percent polymeric color, and antioxidant capacity of black raspberries that were individually quick-frozen (IQF), canned-in-syrup, canned-in-water, pureed, and juiced (clarified and nonclarified). Total monomeric anthocyanins, percent polymeric color, and $ORAC_{FL}$ were determined 1 d postprocessing and after 1, 3, and 6 mo of storage. Thermal processing resulted in marked losses in total anthocyanins ranging from 37% in puree to 69% to 73% in nonclarified and clarified juices, respectively, but only the juices showed substantial losses (38% to 41%) in $ORAC_{FL}$. Storage at 25 °C of all thermally processed products resulted in dramatic losses in total anthocyanins ranging from 49% in canned-in-syrup to 75% in clarified juices. This coincided with marked increases in percent polymeric color values of these products over the 6-mo storage. $ORAC_{FL}$ values showed little change during storage, indicating that the formation of polymers compensated for the loss of antioxidant capacity due to anthocyanin degradation. Total anthocyanins and $ORAC_{FL}$ of IQF berries were well retained during long-term storage at –20 °C.

Keywords: anthocyanins, antioxidant capacity, black raspberries, polymeric color, processing

Introduction

Many epidemiological studies inversely relate fruit and vegetable consumption to the incidence of chronic diseases such as cancer, cardiovascular disease, and stroke (Hertog and others 1996; Joshipura and others 2001; Maynard and others 2003). The lower mortality rates have been attributed to the high concentration of phytochemicals, especially polyphenolics, compounds present in fruits and vegetables in abundant quantities with demonstrated antioxidant potential (Bravo 1998) as well as numerous other health-promoting properties (Hou 2003; Liu and Finley 2005).

Polyphenolics are secondary metabolites in plants that protect against biotic and abiotic stresses. Anthocyanins are a major class of polyphenolics that provide the vibrant red, purple, and blue colors to many fruits, including berries. Black raspberries (*Rubus occidentalis* L.) are highly concentrated in anthocyanin pigments compared to other fruit, and have significant antioxidant capacity according to the oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power assays (Moyer and others 2002). The high levels of anthocyanins, or possibly other polyphenolics, in black raspberries may play an important role in anticancer effects observed in various cancer models (Harris and others 2001; Kresty and others 2001; Casto and others 2002; Huang and others 2002).

Black raspberries, like other berries, not only are available fresh, but are also available for consumption in fresh, frozen, and thermally processed (jellies, jams, juices, and purees) forms. Several studies have investigated the effects of processing on berry anthocyanins. Freezing and subsequent frozen storage have shown to have minimal affect on red raspberry anthocyanins (De Ancos and others 2000; Mullen and others 2002). However, significant losses of anthocyanins have been observed in blueberry juices (Skrede and others 2000; Lee and others 2002; Rossi and others 2003; Srivastava and others 2007), raspberry puree (Ochoa and others 1999) and jams (Garcia-Viguera and others 1998), and strawberry jams (Garcia-Viguera and others 1999; Ngo and others 2007), canned fruit (Ngo and others 2007), juice (Klopotek and others 2005), and nectar (Klopotek and others 2005).

Processing methods varying in the number of processing steps, heating temperature, and duration can markedly affect the anthocyanin content and antioxidant capacity of fruit. However, information is limited on how different processing methods and long-term storage affect black raspberry's nutritional quality. This information is needed for consumers who wish to incorporate higher levels of bioactive compounds into their diet, and processors who desire to retain or, possibly boost, levels of bioactive compounds in their products.

This study evaluated changes in total monomeric anthocyanins, percent polymeric color, and antioxidant capacity due to thermal processing and storage of juiced, canned-in-water (CW), canned-in-syrup (CS), and pureed blackberries and storage of individually quick-frozen (IQF) black raspberries.

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Materials and Methods

Black raspberry samples

Black raspberries (cv. Munger) harvested at the fully ripe stage (shiny black) were obtained from a commercial grower in Oregon in July 2005.

Freezing process and storage

Approximately 5 kg of berries were spread uniformly on stainless steel trays and placed in a Harris Classic Ultralow freezer (Kendro Laboratory Products, Asheville, N.C., U.S.A.) at –70 °C for 12 h (for IQF). The frozen fruit were then placed in Ziploc® freezer bags (0.044-mm thickness) (S.C. Johnson & Son Inc., Racine, Wis., U.S.A.), sealed, and stored at –20 °C. The remaining berries were stored at –20 °C prior to processing.

Canned-in-water (CW) and canned-in-syrup (CS) processing

Black raspberries were canned by the protocol described for blackberries (Downing 1996). Frozen berries (278 g) were added to 303 × 406 cans. Syrup was prepared by adding Sweetose 4300 corn syrup (Tate and Lyle, London, U.K.) to boiling water to reach a final °Brix reading of 40°. Boiling syrup (for CS cans) or water (for CW cans) was added to the cans to the brim and cans were exhausted for 4 min in a steam box (American Sterilizer Co., Erie, Pa., U.S.A.) at 87.8 to 93.3 °C. The cans were then sealed, immersed in boiling water for 15 min, and stored at 25 °C. The canned products were evaluated by 2 methods. In the 1st method, the entire contents of the can (berries + water, berries + syrup) were blended and analyzed. The 2nd method involved separation of the berries and liquid canning media and analysis of each fraction. The berry weight and volume of the liquid canning media of each can were determined after draining the water or syrup from the berries through a nr 8 sieve screen for 3 min.

Juice processing

A diagrammatic scheme of juice processing steps and sampling points is shown in Figure 1. Frozen berries were simultaneously heated and mixed with a Mixco Batch mixer (Avon, N.Y., U.S.A.) in a large steam kettle until the berry mash reached 95 °C. It was held at 95 °C for 3 min and allowed to cool to 40 °C. Depectinization of the mash was performed by adding 0.0827 mL/kg of Pectinex Smash® (Novozyme, Bagsvaerd, Denmark) and incubating the mash for 1 h. Negative alcohol precipitation test was used as an indication of complete depectinization. This test involved the addition of 15 mL of 95% ethanol containing 1% HCL to 5 mL of juice. The visual lack of precipitate and gel formation was indicative of complete depectinization. Following the enzymatic treatment, the mash was pressed in a 25-L Enrossi bladder press (Enoagricol Rossi s.r.l., Calzolaro, Italy) and the juice and press-cake were isolated. Half of the juice was clarified by centrifugation for 10 min at 6000 × *g* in a model CRU-5000 centrifuge (Damon/IEC Div., Needham, Mass., U.S.A.), while the other half received no clarification treatment. Both clarified and nonclarified juices were filled into 6 oz glass bottles and heated in a steam box (American Sterilizer Co.) until the juice temperature reached 90 °C. The bottle caps were tightened and the juices were allowed to cool overnight. Juice samples were stored in the dark at 25 °C.

Puree processing

Frozen berries were thawed and then homogenized using a commercial blender. Blended berries were immediately added to the

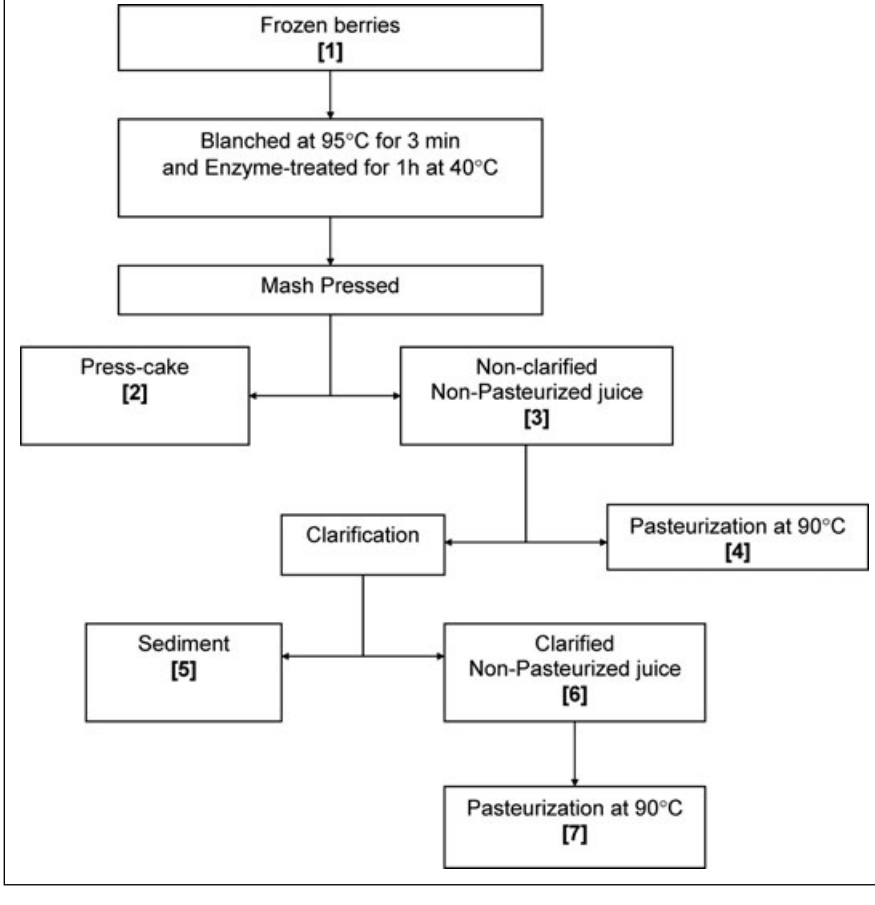

Figure 1 — Diagrammatic scheme of the juice processing with the sampling points indicated (1 to 7).

steam kettle and heated to a temperature of approximately 95 °C. The puree was cooled and Sweetose 4300 corn syrup was added to the puree until 18° Brix was attained. The puree was subsequently heated to 92.8 °C and added to 4-oz canning jars (Ball Corp., Muncie, Ind., U.S.A.). After sealing, the jars were immersed in boiling water for 15 min, cooled in cold water to 38 °C, and stored in the dark at 25 °C.

Extraction of anthocyanins

Prior to extraction of anthocyanins, fresh and IQF fruit, berries isolated from canned products (water and syrup), and entire contents of canned samples (berries + water and berries + syrup) were blended for 1 min on high speed in a commercial food processor. Puree and juice samples required no pre-extraction step.

Blended samples (10 g) of each product were homogenized with 20 mL of methanol/water/formic acid [60:37:3 (v/v/v)] by a Euro Turrax T18 Tissuemizer (Tekmar-Dohrman Corp., Mason, Ohio, U.S.A.). The samples were filtered through Miracloth (Calbiochem, LaJolla, Calif., U.S.A.), the filter cakes isolated, and the extraction repeated. The filtrates were adjusted to a final volume of 100 mL with extraction solvent.

HPLC analysis of monomeric anthocyanins

Sample extracts (4 mL) were dried using a Speed Vac® concentrator (ThermoSavant, Holbrook, N.Y., U.S.A.) and resuspended in 3 mL of an aqueous 3% formic acid solution. The anthocyanin analysis by HPLC was performed according to the method of Cho and others (2004) with a 250 × 4.6 mm Symmetry C_{18}® column (Waters Corp., Milford, Mass., U.S.A.). The mobile phase consisted of a binary gradient of 5% formic acid (A) and 100% methanol (B). The flow rate was 1 mL/min with a linear gradient from 2% B to 60% B over 60 min. The anthocyanin peaks were quantified at 510 nm using a Waters Model 996 photodiode array detector (Milford). All anthocyanins were quantified as cyanidin-3-glucoside equivalents (C3Gluc) using an external calibration curve of the standard (Polyphenols Laboratories AS, Sandnes, Norway) ranging from 5 to 125 μg/mL. Total anthocyanins were calculated as the sum of individual anthocyanin glycosides with results expressed as milligrams per 100 g fresh-berry weight.

Polymeric color analysis

Percent polymeric color was determined using the method described by Giusti and Wrolstad (2005). Sample extracts were diluted with water to have an absorbance reading between 0.5 and 1.0 at 512 nm when evaluated by an 8452A Diode Array Spectrophotometer (Hewlett Packard, Palo Alto, Calif., U.S.A.). For analysis, 0.2 mL of 0.90 M potassium metabisulfite was added to 2.8 mL diluted sample (bisulfite bleached sample) and 0.2 mL of DI water was added to 2.8 mL diluted sample (nonbleached, control sample). After equilibrating for 15 min, but not more than 1 h, samples were evaluated at $\lambda = 700$, 512, and 420 nm. Color density was calculated using the control sample according to the following formula:

$$\text{Color density} = [(A_{420\,\text{nm}} - A_{700\,\text{nm}}) + (A_{512\,\text{nm}} - A_{700\,\text{nm}})] \times \text{dilution factor}$$

Polymeric color was determined using the bisulfite-bleached sample using the following formula:

$$\text{Polymeric color} = [(A_{420\,\text{nm}} - A_{700\,\text{nm}}) + (A_{512\,\text{nm}} - A_{700\,\text{nm}})] \times \text{dilution factor}$$

Percent polymeric color was calculated using the formula:

$$\%\ \text{Polymeric color} = (\text{polymeric color/color density}) \times 100$$

$ORAC_{FL}$ analysis

The hydrophilic oxygen radical absorbing capacity (ORAC) assay using fluorescein as fluorescent probe ($ORAC_{FL}$) was carried out on a FluoStar Optima microplate reader (Biomedical Solutions Inc., Stafford, Tex., U.S.A.) as described by Prior and others (2003). Extracts were diluted 1000-fold in phosphate buffer (pH 7) prior to analysis. The final $ORAC_{FL}$ values were calculated using the regression equation between the Trolox or sample concentration and net area under the fluorescence curve. Data are expressed as micromoles TE per gram fresh-berry weight.

Conversion of data to fresh-berry weight

(1) For blended canned samples, juices, and purees, the monomeric anthocyanin and $ORAC_{FL}$ values were converted to fresh-berry weight using the following calculation:

$$C_{\text{product}} \times R = C_{\text{berry}}$$

where C_{product} = concentration of product, R = ratio of the mass of product produced to the mass of fresh berry, and C_{berry} = concentration based on fresh-berry weight. This conversion allowed for concentration and dilution effects to be accounted for and comparison of all products on an equivalent basis.

(2) For canned berries and liquid canning media (water or syrup), all values were determined as total mass present in the can. The following calculation was used:

$$C_{\text{sample}} \times M_{\text{berry or liquid canning media}} = T_{\text{can}}$$

where C_{sample} = concentration of sample, $M_{\text{berry or liquid canning media}}$ = mass of berry or liquid canning media in the can, and T_{can} = total present in the can. This calculation allowed for canning media (syrup or water) and berry distribution with processing and storage to be determined.

Statistical analysis

All data were reported as means ± standard error of 5 samples taken from each product at each sampling time (fresh, 1 d, and 1, 3, and 6 mo). Effects of juice processing steps on monomeric anthocyanins, percent polymeric color, and antioxidant capacity were analyzed by one-way analysis of variance (ANOVA) (JMP software version 6.0, Cary, N.C., U.S.A.). Significant differences ($P \leq 0.05$) between means were determined by Student's t-test.

Results and Discussion

Processing and storage effects on monomeric anthocyanins and polymeric color

The effects of processing and storage on total monomeric anthocyanins and percent polymeric color values of IQF, berries CW, berries CS, nonclarified juices, clarified juices, and black raspberry purees are shown in Figure 2.

IQF. Individual quick freezing and storage of black raspberries had a minor effect on the total anthocyanins and percent polymeric color. The slight increase in total anthocyanins after the 6-mo storage may be due to a concentration effect due to moisture loss, or enhanced extraction of the anthocyanins due to tissue softening. Our results are consistent with those of previous studies showing that anthocyanins in red raspberries were retained well during frozen storage (de Ancos and others 2000; Mullen and others 2002).

Canned. Processing of berries canned in water or syrup resulted in total anthocyanin losses of 42% and 51%, respectively, when the entire contents of the can were blended and analyzed. The levels of anthocyanins continued to decline in a linear fashion during storage, with losses of 76% and 75% observed in berries canned-in water or syrup, respectively, after a 6-mo storage. Canning had a minor impact on percent polymeric color values, but anthocyanins were extensively polymerized during the 6-mo storage, with percent polymeric color values increasing from 7.9% (fresh) to 21% in berries canned in water and 7.9% (fresh) to 22% in berries canned in syrup. Consistent with our findings, Ngo and others (2007) reported that total anthocyanins in strawberries canned in 20 °B syrup declined 69% over 60-d room temperature storage, during which time percent polymeric color values increased from 7.2% to 33.3% and 27.4% in fruit and syrup, respectively. In contrast to our results, Chaovanalkit and Wrolstad (2004) reported that anthocyanins in Bing cherries increased slightly after canning; however, consistent with our findings, they observed a major loss in anthocyanins over a 5-mo storage, during which time percent polymeric color values increased markedly in both cherry and syrup fractions. They also observed an increase in percent polymeric color values (13% to 40% in cherries and 13% to 35% in syrup) over the 5-mo storage. The distribution of anthocyanins in berries and liquid canning medium (water and syrup) was determined. In berries CW, 25% to 37% of anthocyanins diffused out of the fruit into the aqueous medium over the 6-mo storage, while 63% to 75% of the anthocyanins were retained in the berries (Figure 3). In berries CS, 30% to 36% of the anthocyanins diffused out of the fruit into the syrup, while 64% to 70% were retained in the berries over the 6-mo storage (Figure 3). Leaching of anthocyanins in black raspberries in response to canning and storage was less than previous reported values for canned Bing cherries (50% diffusion; Chaovanalikit and Wrolstad 2004) and strawberries (60% diffusion; Ngo and others 2007). This discrepancy may be attributed to differences in fruit structure and localization of anthocyanins. In black raspberries, anthocyanins are localized in drupelets, while in cherries and strawberries, anthocyanins are located predominantly in epidermal tissue (Chaovanalikit and Wrolstad 2004; Aaby and others 2005). Presumably, the anthocyanins located in epidermal tissues are more susceptible to leaching into the liquid canning medium.

Juices. Changes in total anthocyanins were evaluated during different steps in juice processing (Table 1). Approximately 16% of the original concentration of anthocyanins was lost to the press-cake during juice pressing, and 1.3% of the original concentration of anthocyanins was removed as sediment in the juice clarification step. Taking into account the 38.2% retention of anthocyanins in nonclarified, nonpasteurized juice and the 16.4% retained in the press-cake (total = 54.6%), it is apparent that 45.4% of the original anthocyanins were degraded during the maceration, blanching, and depectinization treatments. A similar mass balance for clarified juice, 34.4% retention in clarified, nonpasteurized juice, 16.4% retention for press-cake, and 1.3% retention for sediment (total = 52.1%), indicates that 47.9% of the original anthocyanins were degraded prior to pasteurization. Pasteurization of nonclarified and clarified juices resulted in anthocyanin losses of 19% and 23%, respectively. Consistent with our findings, significant losses (up to 65%) of anthocyanins during the primary steps of juice processing (thawing, crushing, depectinization, and pressing) were previously reported for blueberries (Skrede and others 2000; Lee and others 2002; Rossi and others 2003; Srivastava and others 2007), and strawberries (Klopotek and others 2005). The extensive losses of anthocyanins in our study were most likely due to enzymatic degradation that occurred during the fruit maceration step prior to blanching.

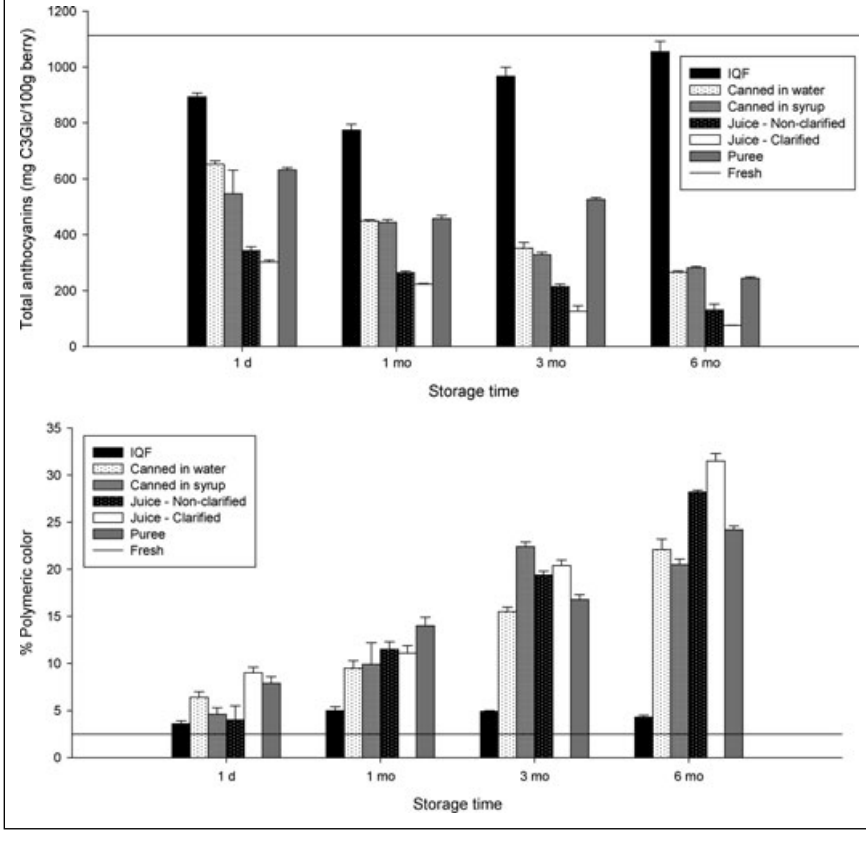

Figure 2—Total monomeric anthocyanins (A) and percent polymeric color (B) of IQF black raspberries as affected by storage at –20 °C, and thermally processed black raspberries as affected by storage at 25 °C. Bars represent ± SEM (n = 5).

Polyphenoloxidase (PPO) has been shown to play an important role in enzymatic browning of blueberry anthocyanins (Kader and others 1997a, 1997b), but the enzyme is readily destroyed by heating (Skrede and others 2000).

The percent polymeric color values did not change much during juice processing steps (Table 1), with the exception of the press-cake value, which was 5% higher than the value for frozen fruit used as the raw material. It was possible that significantly higher levels of anthocyanins were retained in the press-cake, but the compounds were extensively polymerized and not detected by HPLC analysis.

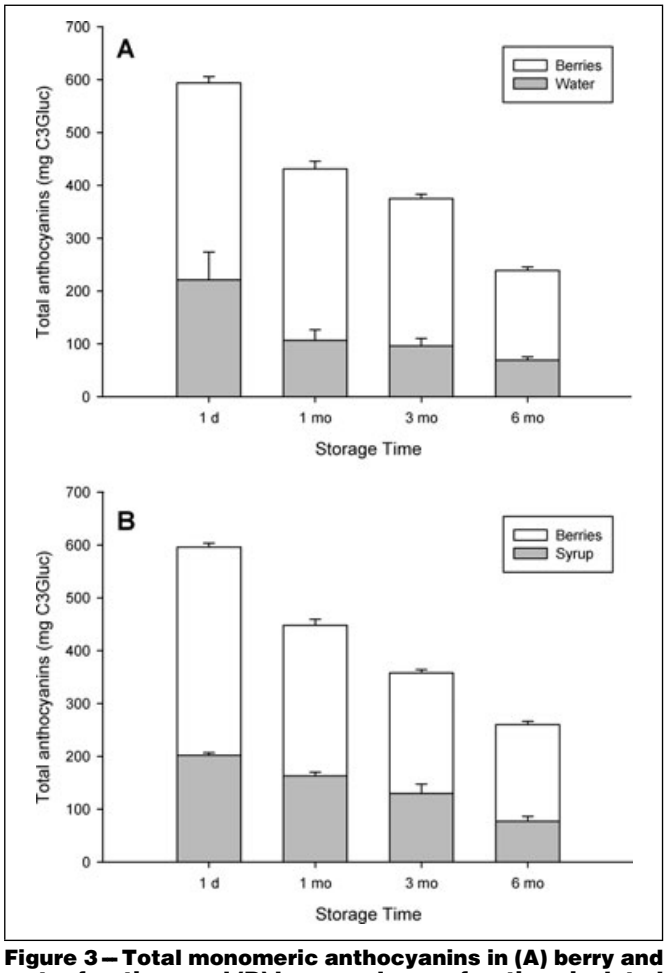

Figure 3 — Total monomeric anthocyanins in (A) berry and water fractions and (B) berry and syrup fractions isolated from canned black raspberries as affected by storage at 25 °C. Bars represent ± SEM (n = 5).

The effects of storage on total anthocyanins and percent polymeric color of nonclarified and clarified juices are shown in Figure 2. Levels of total anthocyanins decreased linearly during storage, with losses of 62% and 75% observed for nonclarified and clarified juices from 1 d to 6 mo of storage. After 6 mo of storage, the nonclarified and clarified juices contained only 13% and 8%, respectively, of anthocyanins present in fresh berries. Similar to other thermally processed products, anthocyanin losses during storage were accompanied by linear increases in percent polymeric color values, which increased from 8.6% (fresh) to 28% in nonclarified juices, and from 8.6% (fresh) to 32% in clarified juices over 6 mo of storage.

The large increases in polymeric color and corresponding loss of anthocyanins with storage in all thermally processed products may be due to several factors, including residual enzyme activity as well as condensation reactions of anthocyanins with other phenolics. We detected PPO and peroxidase (POD) activities in extracts of fresh black raspberries using catechol and 3,3′,5′,5 tetramethylbenzidine as substrates (data not shown). Although POD and PPO have been shown to cause anthocyanin degradation in the presence of cofactors such as chlorogenic acid for PPO (Kader and others 1997b) and chlorogenic acid + H_2O_2 for POD through chlorogenoquinone mediated reactions (Kader and others 2002), we did not detect any residual PPO or POD activity in the thermally processed products (data not shown). A more plausible mechanism involves condensation reactions of anthocyanins with other phenolic compounds, including flavan-3-ols or polyflavan-3-ols (Reed and others 2005), that can be mediated by acetaldehyde (Es-Safi and others 1999) and furfural (Es-Safi and others 2000), or occur via direct anthocyanin-tannin reactions (Remy and others 2000). Phenolic acids such as ferulic and syringic acid have also been shown to complex with anthocyanins in strawberry and raspberry juices (Rein and others 2005). However, we did not observe any new peaks in the HPLC chromatograms indicative of degradation compounds or the formation of pyranoanthocyanins. We suspect that the condensation products between anthocyanins and other phenolics were of large molecular weight and were either not separated on the reverse phase C18 column or possibly removed by the filtering step prior to HPLC analysis.

Puree. Processing of puree resulted in a 37% loss in total anthocyanins. We suspect that the marked loss of anthocyanins was

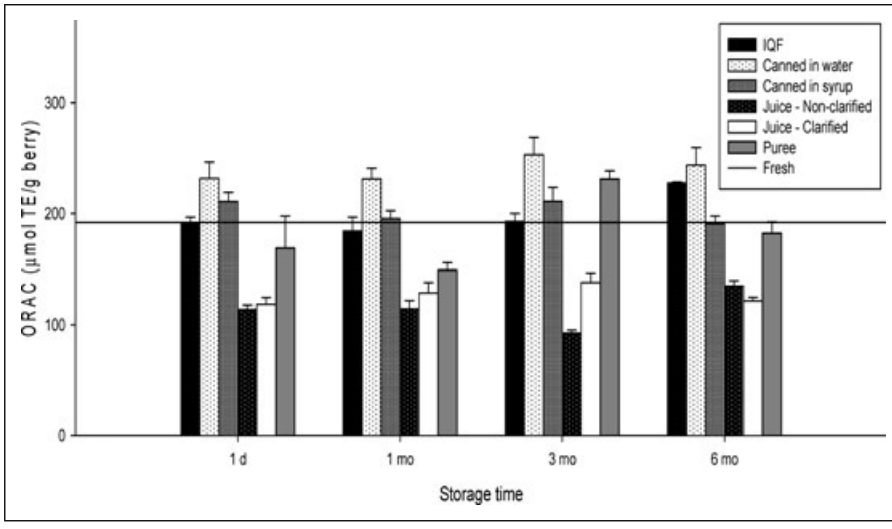

Figure 4 — $ORAC_{FL}$ of IQF black raspberries as affected by storage at –20 °C, and thermally processed black raspberries as affected by storage at 25 °C. Bars represent ± SEM (n = 5).

Table 1 — Total monomeric anthocyanins, percent polymeric color, and $ORAC_{FL}$ values through out juice processing with each processing step corresponding to steps indicated in Figure 1.

Processing step	Total monomeric ACYs (mg/100 g berry)	% Polymeric color	$ORAC_{FL}$ (μmol TE/g berry)
[1]Fresh	1113.1 ± 30.7[B]a[C]	8.6 ± 0.6c	192.2 ± 9.3a
[2]Press-cake	182.9 ± 13.0e	13.5 ± 0.4a	18.4 ± 1.0d
[3]Juice, NC[A], NP[A]	425.1 ± 9.9b	9.2 ± 0.5c	94.9 ± 5.6c
[4]Juice, NC, P[A]	342.9 ± 14.2c	11.5 ± 0.8b	118.6 ± 5.9b
[5]Sediment	14.0 ± 0.6f	5.9 ± 0.5b	7.3 ± 0.2e
[6]Juice, C[A], NP	393.5 ± 5.4b	11.1 ± 0.7b	91.3 ± 1.1c
[7]Juice, C, P	302.4 ± 7.2d	9.0 ± 0.6c	113.6 ± 4.2b

[A]NC = nonclarified; NP = nonpasteurized; P = pasteurized; C = clarified.
[B]Values represent means ± standard error ($n = 5$).
[C]Means within columns with different letters are significantly different ($P \leq 0.05$).

the result of enzymatic degradation, which occurred from the time berries were thawed and pureed until the puree was heated to 95 °C. Levels of total anthocyanins continued to decrease during room temperature storage, with a 76% loss observed after a 6-mo storage. The loss of total anthocyanins was accompanied by increased polymeric color values, which increased from 8.6% (fresh fruit) to 24% after a 6-mo storage. The results indicate that anthocyanins were extensively polymerized during storage. Anthocyanin losses were also accompanied by increased percent polymeric color values in raspberry pulp stored at different temperatures (Ochoa and others 1999).

Processing and storage effects on $ORAC_{FL}$

The effects of processing and storage on the antioxidant capacity of black raspberry products were evaluated by the $ORAC_{FL}$ assay, which measures the scavenging capacities of peroxyl radicals (Figure 4). The $ORAC_{FL}$ value of fresh Munger black raspberries (192.2 μmol TE/g) is exceptionally high compared to other antioxidant rich fruit such as low-bush blueberry (92.1 μmol TE/g), cranberry (92.6 μmol TE/g), and black plums (73.0 μmol TE/g) (Wu and others 2004), which may be attributed to the high levels of anthocyanins (1113 mg/100 g) found in the fruit.

IQF. The $ORAC_{FL}$ values remained stable postfreezing and throughout the 3-mo storage, but increased by 18% at 6 mo, presumably due to higher anthocyanin content as a result of moisture loss, or enhanced extraction due to tissue softening.

Canned. Despite significant losses in total anthocyanins, $ORAC_{FL}$ values of berries canned in syrup or water (whole can blended) did not change in response to processing. $ORAC_{FL}$ of berries canned in syrup remained stable during storage, while values for berries canned in water remained stable up to 1 mo of storage, but increased by 32% and 27% after 3- and 6-mo storage. The retention in antioxidant capacity during storage conflicts with the marked losses observed in total anthocyanins, and may be explained by the formation of anthocyanin polymers (Tsai and Huang 2004; Tsai and others 2004), which compensated for the loss of monomeric anthocyanins. Our results are consistent with those of Chaovanalikit and Wrolstad (2004), who reported that ORAC and percent polymeric color values of canned cherries increased after a 5-mo storage at 22 °C.

Juices. Changes in antioxidant capacity were evaluated during different steps in juice processing (Table 1). Approximately 10% of the original antioxidant capacity was lost to the press-cake during juice processing, and 4% of the original antioxidant capacity was removed as sediment in the juice clarifications step. Prior to pasteurization, only 49% and 48% of the original antioxidant capacity were retained in nonclarified and clarified juices, respectively. The major losses in antioxidant capacity during juice processing were consistent with the marked (45% to 47%) losses of total anthocyanins observed. Although pasteurization resulted in a 20% loss in total anthocyanins, the antioxidant capacity of nonclarified and clarified juices increased by 25% and 24%, respectively, in response to pasteurization. The increase in antioxidant capacity may be due to the formation of Maillard reaction products in response to thermal treatment (Yilmaz and Toledo 2005). The $ORAC_{FL}$ values of nonclarified and clarified juices remained stable over the 6-mo storage, despite marked losses of total anthocyanins. As observed in other thermally processed products, the antioxidant capacity of polymeric anthocyanins formed during storage (Tsai and Huang 2004; Tsai and others 2004) likely compensated for the loss of antioxidant capacity as a result of monomeric anthocyanin degradation.

Puree. $ORAC_{FL}$ values remained relatively stable in response to processing and throughout storage, despite significant losses in total anthocyanins, although samples obtained at 3 mo had higher values than samples taken at 1 d and 1 mo. The retention of $ORAC_{FL}$ during storage and apparent increase at 3 mo may reflect antioxidant contribution by anthocyanin polymers (Tsai and Huang 2004; Tsai and others 2004).

Conclusions

Processing black raspberries into various forms resulted in significant losses of monomeric anthocyanins, most notably in nonclarified and clarified juices, which retained only 31% and 27% of the monomeric anthocyanins present in fresh berries. However, freezing had a minimal effect on anthocyanin retention. Monomeric anthocyanins were extensively degraded during storage in all thermally processed products (canned, juices, and purees), with less than 25% of the original total anthocyanins present in the processed products after 6 mo. In canned products, significant amounts of monomeric anthocyanins (25% to 37%) leached out of the berries into the liquid canning medium. Losses of monomeric anthocyanins during storage were accompanied by increased polymeric color values, indicating that monomeric anthocyanins were extensively polymerized during storage. Despite marked losses of monomeric anthocyanins in all thermally processed products, $ORAC_{FL}$ values changed little during storage, suggesting that polymeric compounds formed during storage compensated for the loss of antioxidant capacity due to degradation of monomeric anthocyanins. More research is needed to identify the anthocyanin polymers and to determine their bioavailability *in vivo*.

Acknowledgments

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