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

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Blackberries are a rich source of polyphenolics, particularly anthocyanins, that may contribute to the reduced risk of chronic disease; however, as with most berries, the fresh fruit are only seasonally available. With most of the blackberries consumed as frozen or in thermally processed forms after long-term storage, the purpose of this study was to evaluate the effects of processing and 6 months of storage on the anthocyanins and antioxidant capacity of blackberries that were individually quick-frozen (IQF), canned-in-syrup, canned-in-water, pureed, and juiced (clarified and nonclarified). Monomeric anthocyanins, percent polymeric color, and antioxidant capacity by oxygen radical absorbance capacity ($ORAC_{FL}$) and photochemiluminescence (PCL) were determined postprocessing (1 day) and after 1, 3, and 6 months of storage. Processing resulted in increases in polymeric color values (up to 7%) and losses in monomeric anthocyanins (up to 65%). For most products, processing also resulted in losses in antioxidant capacity (by $ORAC_{FL}$ and PCL). Storage at 25 °C of all processed products resulted in dramatic losses in monomeric anthocyanins with as much as 75% losses of anthocyanins throughout storage, which coincided with marked increases of percent polymeric color values of these products over 6 months of storage. There were no changes in $ORAC_{FL}$ or PCL for processed products throughout long-term storage. No significant changes in antioxidant capacity or anthocyanin content were observed in IQF fruit during long-term storage at −20 °C.

KEYWORDS: Blackberries; anthocyanins; polymeric color; antioxidant; processing and storage

INTRODUCTION

Diets rich in fruits and vegetables have been associated with reduced risk of chronic diseases, such as cancer, cardiovascular disease, and stroke, in many epidemiological studies (*1–3*). 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 (*4, 5*), as well as other health-promoting properties (*5*).

Polyphenolics are ubiquitous plant components, primarily secondary metabolites, that protect against biotic and abiotic stresses. Significant sources of polyphenolics include highly pigmented blueberries, raspberries, and blackberries. Flavonoids and tannins are the major polyphenolics present in berry fruits. Blackberries are highly concentrated in anthocyanin pigments and have significant antioxidant capacity according to the oxygen radical absorbance capacity (ORAC) (*6, 7*), Trolox equivalent antioxidant capacity (TEAC) (*8*), and ferric reducing antioxidant power (FRAP) (*7*) assays.

Blackberries, like other berries, are not only available fresh but also widely distributed as frozen and thermally processed (jellies, juices, purees, cobblers, and pies) products. The effects of freezing on the vitamin and nutritional contents of vegetables have been documented (*9, 10*), but data on the effects of thermal treatments and subsequent storage on polyphenolics in fruits and vegetables are limited. However, several studies report that processing may result in significant losses of water-soluble phenolics (*11–14*), especially anthocyanins (ACYs), which adversely affect the color and nutritional quality of the products. According to Nicoli et al. (*15*), the antioxidant capacity of foods may be affected by processing in several ways, including losses of water-soluble antioxidants (such as phenolics), alterations in the compounds that improve or reduce the antioxidant capacity of the plant constituents, interactions with other compounds that alter the antioxidant activity, or formation of novel compounds by Maillard or other reactions that affect antioxidant activity.

With the diverse availability and potential sources of phytochemical changes throughout processing and storage, a

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thorough evaluation of changes in phenolics and antioxidant capacity due to processing and storage is important for the consumer, particularly in regard to recommended daily servings of fruits. This information is also of interest to processors who wish to retain or possibly boost levels of phytochemicals in their products. With the demonstrated antioxidant capacity of blackberries and the significant concentrations of ACYs, blackberry fruits offer an excellent model for the evaluation of the effects of processing and storage on ACYs and antioxidant capacity. The purpose of this study was to evaluate changes in total monomeric ACYs, percent polymeric color, and antioxidant capacity due to thermal processing and storage of several blackberry products.

MATERIALS AND METHODS

Materials. Blackberries (cv. Apache) harvested at the fully ripe stage (shiny black) were obtained from the Cox Berry Farm (Clarksville, AR) in July 2005. Approximately 5 kg of berries was spread uniformly on stainless steel trays and placed in a Harris Classic Ultralow freezer at −70 °C for 12 h (for IQF). The frozen fruits were then placed in ziplock bags and stored at −20 °C. The remaining berries were stored at −20 °C prior to processing.

Juice Processing. Frozen berries were simultaneously heated and mixed with a Mixco Batch mixer (Avon, NY) 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. A negative alcohol precipitation test was used as an indication of complete depectinization. Following enzymatic treatment, the mash was pressed in a 25 L Enrossi bladder press (Enoagricol Rossi s.r.l., Calzolaro, Italy), and the juice and presscake were isolated. Half of the juice was clarified by centrifugation for 10 min at 6000*g* in a model CRU-5000 centrifuge (Damon/IEC Division, Needham, MA), whereas the other half received no clarification treatment. Both clarified and nonclarified juices were aliquotted into 6 oz glass bottles and heated in a steam box (American Sterilizer Co., Erie, PA) 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.

Canned-in-Water and Canned-in-Syrup Processing. Blackberries were canned according to protocol described by Downing (*16*). Frozen berries (278 g) were added to 303 × 406 cans. Syrup was prepared by adding Sweetose (Tate and Lyle, London, U.K.) to boiling water to reach a final °Brix reading of 40°. Boiling syrup (for canned-in-syrup cans) or water (for canned-in-water cans) was added to the cans to the brim, and cans were exhausted for 4 min in a steam box (American Sterilizer Co.) at 87.8–93.3 °C. The cans were then sealed, immersed in boiling water for 15 min, and stored at 25 °C. For extraction, products were blended (with berries and brine) or separated into berries and brine and extracted separately. For each can, the berry weight and brine volume were determined after brine had been drained from the berries through a no. 8 sieve screen for 3 min.

Puree Processing. Frozen berries were allowed to thaw and homogenized using a commercial household blender. Blended berries were immediately added to the steam kettle and heated to a temperature of ∼95 °C. The puree was cooled, and Sweetose 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, IN). 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.

Sample Extraction. Pureed samples (5 g) 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, OH). The samples were filtered through Miracloth (Calbiochem, La Jolla, CA), the filter cakes were isolated, and the extraction was repeated. The filtrates were adjusted to a final volume of 50 mL with extraction solvent.

HPLC Analysis of Anthocyanins. Sample extracts (4 mL) were dried using a Speed Vac concentrator (ThermoSavant, Holbrook, NY) and resuspended in 2 mL of 3% formic acid. The ACY analysis by HPLC was performed according to the method of Cho et al. (*17*) with a 250 × 4.6 mm Symmetry C_{18} column (Waters Corp., Milford, MA). The mobile phase consisted of a binary gradient of 5% formic acid (A) and 100% methanol (B). The flow rate was 1.0 mL/min with a linear gradient from 2 to 60% B over 60 min. The ACY peaks were quantified at 510 nm using a Waters model 996 photodiode array detector. All ACYs (cyanidin 3-glucoside, cyanidin 3-rutinoside, cyanidin 3-xyloside, cyanidin 3-malonylglucoside, and cyanidin 3-dioxalylglucoside) were quantified as cyanidin-3-glucoside equivalents with total monomeric ACY results expressed as milligrams per 100 g of original berry.

Polymeric Color Analysis. Percent polymeric color was determined using the method described by Giusti and Wrolstad (*18*). Sample extracts were diluted with water in order 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, CA). For analysis, 0.2 mL of 0.90 M potassium metabisulfite was added to 2.8 mL of diluted sample (bisulfite-bleached sample) and 0.2 mL of DI water was added to 2.8 mL of 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 $ORAC_{FL}$ assay was utilized to evaluate the ability of the extracts to scavenge peroxyl radicals. Hydrophilic $ORAC_{FL}$ analysis was performed on a dual pump BMG FLUOstar Optima plate reader (Durham, NC) as described previously (*6*). Results were calculated on the basis of differences in areas under the fluorescence decay curve between the blank, samples, and standards. The standard curve was obtained by plotting the four concentrations of Trolox (6.25, 12.5, 25, 50 μM) equivalents (TE) against the net area under the curve (AUC) of each standard. Final $ORAC_{FL}$ values were calculated using the regression equation between TE concentration and AUC and are expressed as micromoles of TE per gram of original berry. It has been previously reported that the coefficient of variation for this method is 3–6% (*6*).

PCL Analysis. The photochemiluminescent (PCL) assay with a Photochem instrument (Analytik Jena AG, Jena, Germany) was used to measure the hydrophilic antioxidant activity of extracts against superoxide radicals generated from luminol, a photosensitizer, when exposed to UV light. Only the hydrophilic assay (labeled "ACW" by the manufacturer) was utilized in this study. A 0.04% luminol solution was prepared by dissolving 0.02 g of luminol in 0.5 mL of 5% NaOH and taken to a final volume of 500 mL with water. For each sample, a 1.0 mL solution of 0.1 M carbonate buffer (pH 10.6) containing 0.1 mM EDTA was diluted with 1.5 mL of water and combined with 25 μL of the prepared luminol solution. Immediately before analysis, Trolox calibration standards prepared in methanol (0.25, 0.50, 0.75, 1.0, and 1.5 nmol) or sample (2.5–20 μL) were added to the solution. The antioxidant activity was estimated by the duration of lag phase over 1–3 min compared with a Trolox standard curve and expressed as micromoles of TE per gram of original berry. Previous work has reported that the PCL assay within-batch variability is <2% and batch-to-batch variability, <5% (*19*).

Calculations. For blended cans, juices, and purees, the monomeric ACYs, $ORAC_{FL}$, and PCL were converted to original berry weight. The following calculation was used:

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

C_{product} = concentration of product, R = ratio of the mass of product produced to the mass of the original berry, and C_{berry} = concentration based on original berry weight.

Table 1. Total Monomeric Anthocyanins and Percent Polymeric Color, $ORAC_{FL}$, and PCL Values for Blended Canned-in-Syrup, Blended Canned-in-Water, and Puree As Affected by Processing (1 Day)[a]

product	total monomeric ACYs (mg/100 g of berry)	% polymeric color	$ORAC_{FL}$ (μmol of TE/g of berry)	PCL (μmol of TE/g of berry)
IQF	248 ± 6.4a	10.5 ± 0.6b	97.2 ± 10.1a	19.5 ± 1.6a
canned-in-syrup	221 ± 4.5b	17.8 ± 1.2a	75.8 ± 2.2b	15.2 ± 0.8b
canned-in-water	204 ± 7.2c	14.2 ± 2.2ab	71.1 ± 1.5b	16.5 ± 1.1ab
puree	180 ± 4.6d	12.4 ± 0.3b	85.0 ± 4.6ab	19.0 ± 0.7a

[a] Values represent means ± standard error ($n = 6$ for monomeric ACYs, percent polymeric color, and PCL; $n = 3$ for $ORAC_{FL}$ and PCL). Means within columns with different letters are significantly different ($p \leq 0.05$).

Table 2. Total Monomeric Anthocyanins, Percent Polymeric Color, $ORAC_{FL}$, and PCL Values Throughout Juice Processing with Each Processing Step Corresponding to Steps Indicated in **Figure 1**

processing step	total monomeric ACYs (mg of C3Gluc/100 g of berry)	% polymeric color	$ORAC_{FL}$ (μmol of TE/g of berry)	PCL (μmol of TE/g of berry)
[1] frozen	248 ± 6.4a	10.5 ± 0.6ef	97.2 ± 10.1a	19.5 ± 1.6a
[2] blanched	163 ± 3.3b	13.2 ± 0.5cd	63.2 ± 5.6b	19.2 ± 0.5a
[3] presscake	75.1 ± 1.5e	22.0 ± 1.4b	46.9 ± 4.6c	12.8 ± 0.4b
[4] juice, NC, NP	129 ± 2.0c	9.2 ± 0.6f	39.6 ± 2.1c	11.5 ± 0.4bc
[5] juice, NC, P	83.6 ± 2.4e	16.5 ± 1.5c	42.9 ± 0.8c	9.4 ± 1.2c
[6] sediment	1.7 ± 0.1f	36.0 ± 2.2a	0.7 ± 0.0d	0.1 ± 0.0c
[7] juice, C, NP	106 ± 4.1d	11.5 ± 0.7def	34.6 ± 3.3c	10.6 ± 0.4bc
[8] juice, C, P	82.0 ± 1.6e	12.3 ± 0.5de	43.8 ± 0.8c	10.1 ± 0.2c

[a] Values represent means ± standard error ($n = 6$ for monomeric ACYs, percent polymeric color, and PCL; $n = 3$ for $ORAC_{FL}$). Means within columns with different letters are significantly different ($p \leq 0.05$). Abbreviations: NC, nonclarified; NP, nonpasteurized; P, pasteurized; C, clarified.

This conversion allowed for concentration and dilution effects to be accounted for and everything was compared on an equivalent basis.

For canned berries and brine, all values were determined as total mass present in the can. The following calculation was used:

$$C_{\text{sample}} M_{\text{berry or brine}} = T_{\text{can}}$$

C_{sample} = concentration of sample, $M_{\text{berry or brine}}$ = mass of berry or brine in the can, and T_{can} = total present in the can.

This calculation allowed for brine and berry distribution with processing and storage to be determined.

Statistical Analysis. Effects of processing and storage on monomeric ACYs, percent polymeric color, and antioxidant capacity were analyzed by one-way analysis of variance (JMP software v. 6.0, Cary, NC). Significant differences ($p \leq 0.05$) between means were determined by analysis of variance.

RESULTS AND DISCUSSION

Processing Effects on Monomeric Anthocyanins and Polymeric Color. In all thermally processed products, the concentration of monomeric ACYs decreased with processing as compared to IQF berries (**Tables 1** and **2**). Juice processing resulted in the greatest losses (~67%), whereas canned products were the least affected by processing with only 17.8 and 10.5% losses for canned-in-water and canned-in-syrup blended (berry + brine) cans, respectively. Thermal processing of purees resulted in 27.4% loss in total monomeric ACYs. Thermal processing also resulted in increases in percent polymeric color values (**Tables 1** and **2**) in all products ranging from an increase of 1.7% for clarified juice to an increase of 7.3% for canned-in-syrup berries. Chaovanalikit and Wrolstad (*20*) reported that polymeric color values increased up to 35% in cherries after canning, but they observed no concomitant losses in monomeric ACYs.

Throughout juice processing the changes in monomeric anthocyanins and polymeric color were evaluated. The processing steps and the sites at which samples were taken are presented in **Figure 1**, and the corresponding data are presented in **Table 2**. Overall significant losses in monomeric ACYs were observed throughout juice processing. Blanching and enzymatic treatment resulted in a

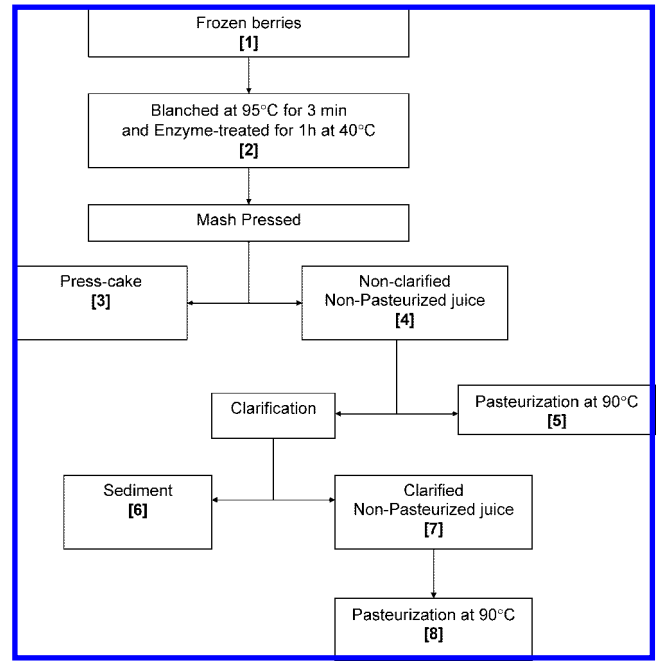

Figure 1. Diagrammatic scheme of juice processing with the sampling points (1−8) identified.

34% loss in the total monomeric ACYs as compared to frozen berries. An additional ~14% of the original concentration of monomeric ACYs was lost to the presscake during juice pressing and the pasteurization step resulted in a further 18% loss in the original concentration of ACYs. Consistent with our findings, significant losses (up to 65%) of monomeric ACYs during juice processing were previously noted for blueberries (*11, 13, 14*) and strawberries (*21*). In contrast, an apparent ACY increase in pasteurized blackberry juice compared to frozen fruit was reported by Rommel et al. (*22*), which was explained by a preferential release of ACYs into the liquid phase.

The percent polymeric color values increased with juice processing (**Table 2** and **Figure 1**); however, the polymers

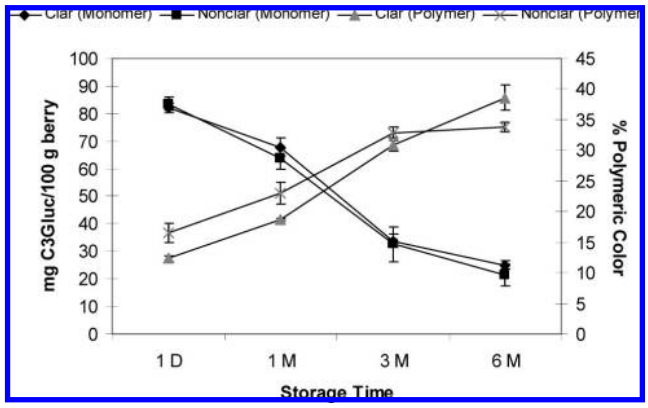

Figure 2. Total monomeric anthocyanins and percent polymeric color values of clarified and nonclarified juices over 6 months of storage at 25 °C. Bars represent standard error of the mean ($n = 6$).

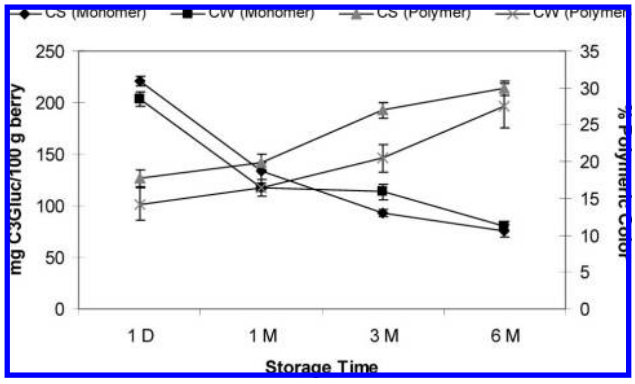

Figure 3. Total monomeric anthocyanins and percent polymeric color values of blended canned-in-syrup and canned-in-water samples over 6 months of storage at 25 °C. Bars represent standard error of the mean ($n = 6$).

appeared to be most concentrated in the presscake and sediment, which may be attributed to residual peroxidase or polyphenol oxidase activity. The decrease in polymeric color of 13.2% from the mash (after blanching and enzyme treatment) to 9.2% in the nonclarified, nonpasteurized juice was likely due to the polymeric ACYs remaining in the presscake (22.0% polymeric color). The pasteurized juices were both higher in polymeric color than the frozen berries (with 16.5 and 12.3% for nonclarified and clarified juices, respectively), likely due to the extent of heating during pasteurization. An increase in polymeric color was also observed in pasteurized blueberry juice compared to frozen fruit used as the raw material (*14*).

Storage Effects on Monomeric Anthocyanins and Polymeric Color. For all thermally treated, shelf-stable products (juices, canned products, and purees), stored at 25 °C, significant losses in monomeric ACYs occurred over 6 months of storage that coincided with increases in percent polymeric color values (**Figures 2**−**4**). There were 69–75% losses in total monomeric ACYs in juices over 6 months of storage with the percent polymeric color increasing from 12.3 to 38.6% and from 16.5 to 33.8% for clarified and nonclarified juices, respectively (**Figure 2**). Significant reductions in monomeric ACYs occurred over 6 months of storage in the other thermally processed products as well with 65.8, 60.6, and 58.4% losses observed for blended canned-in-syrup products, blended canned-in-water products, and purees, respectively (**Figures 3** and **4**). Our results concur with previous studies reporting a decrease in monomeric ACY concentrations of shelf-stable raspberry products over long-term storage at temperatures ≥20 °C (*23, 24*).

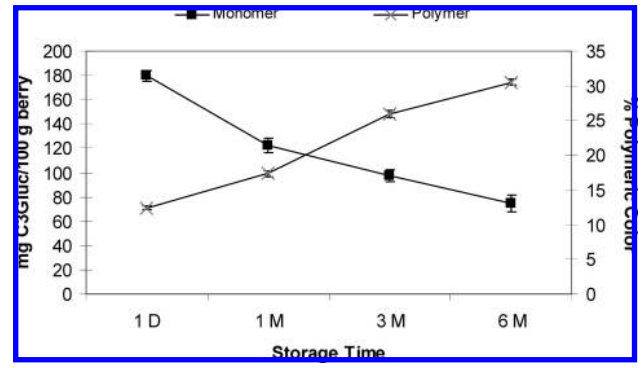

Figure 4. Total monomeric anthocyanins and percent polymeric color values of puree over 6 months of storage at 25 °C. Bars represent standard error of the mean ($n = 6$).

The percent polymeric color values increased from 17.8 to 29.9% and from 14.2 to 27.6% in canned-in-syrup and canned-in-water samples, respectively (**Figure 3**). Over 6 months of storage the puree percent polymeric color values increased from 12.4 to 30.5% (**Figure 4**). All shelf-stable products were susceptible to losses in total monomeric ACYs and significant increases in percent polymeric color values over 6 months of storage, but the juice products were the most significantly affected by storage. This indicates that there may be a protective effect of other components in the fruit generally removed with the presscake during juice processing. Significant increases in polymeric color have also been observed in pasteurized raspberry pulp and canned plums during room temperature storage (*24, 25*).

The large increases in polymeric color with storage and the corresponding loss of monomeric anthocyanins may be the result of residual peroxidase activity. The enzyme is relatively heat stable as compared to polyphenol oxidase and may also regenerate over time, which would allow it to retain activity throughout storage. It has been shown that blueberry peroxidase, in the presence of chlorogenic acid and H_2O_2, rapidly oxidized ACYs into brown pigments with significant absorption between 380 and 420 nm (*26*). Despite nondetectable levels of chlorogenic acid in the blackberry fruit (data not shown), copigmentation or complexation may be the result of other mechanisms besides chlorogenic quinone formation as a result of residual peroxidase activity. For example, it was determined that other phenolic acids (such as ferulic and syringic acid) can complex with ACYs in strawberry and raspberry juices (*27*). Polyphenol oxidase has also been shown to degrade anthocyanins in the presence of chlorogenic acid (*28*) and catechin (*29*) via a similar condensation reaction as peroxidase.

The concentrations of monomeric ACYs and percent polymeric color values were the most stable in IQF fruit over storage (**Figure 5**). There were no significant changes in monomeric ACYs or polymeric color, indicating that frozen storage of intact fruit did not promote losses in ACYs or ACY polymerization. A previous study on raspberries indicated that long-term storage (up to 1 year) did not change the monomeric anthocyanin content significantly (*30*); however, in a study on frozen cherries, stored at −23 °C for 6 months, dramatic increases in polymeric color (from 12.5 to 60.8%) and losses in monomeric anthocyanins (up to 88%) were observed, but no changes were observed in fruit stored at −70 °C (*20, 31*).

Processing Effects on $ORAC_{FL}$ and PCL. The $ORAC_{FL}$ and PCL hydrophilic antioxidant capacity values of thermally processed products decreased significantly postprocessing (**Tables 1** and **2**). Juice processing resulted in 55–56 and

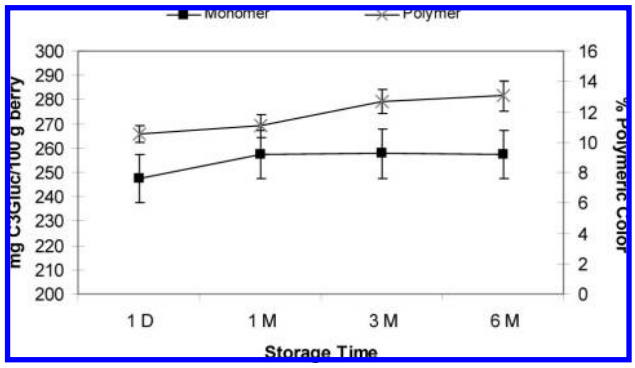

Figure 5. Total monomeric anthocyanins and percent polymeric color values of individually quick-frozen berries over 6 months of storage at −20 °C. Bars represent standard error of the mean ($n = 6$).

48–52% losses in $ORAC_{FL}$ and PCL values, respectively, compared to the original frozen fruit (**Table 2**). The presscake $ORAC_{FL}$ and PCL values were 48 and 65%, respectively, of the original frozen fruit. With such high antioxidant levels in the presscake that overcompensate for losses in the final product, it is possible that the high concentrations of polymers were significant contributors to the antioxidant levels. The pasteurization step did not affect $ORAC_{FL}$ or PCL values of either clarified or nonclarified juice. Because the monomeric ACYs decreased (up to 35% losses) and polymeric color increased (up to 7% increase) with pasteurization in both juices, it is likely that the antioxidant capacity of polymers formed during heating compensated for the loss of antioxidant capacity as a result of anthocyanin degradation.

Losses in $ORAC_{FL}$ for canned-in-syrup and canned-in-water blended samples were 22 and 27%, respectively (**Table 1**), whereas PCL losses for canned-in-syrup and canned-in-water blended samples were 22 and 15%, respectively. However, only the losses observed in the canned-in-syrup blended samples were significant. There were no significant losses in $ORAC_{FL}$ or PCL in pureed products as compared to IQF berries despite significant losses in monomeric anthocyanins; thus, other factors likely contributed to the antioxidant capacity values. This phenomenon is possibly the result of the demonstrated antioxidative activity of polymeric anthocyanins (*32, 33*) and/or Maillard reaction products (*34*) that form in response to heating.

Storage Effects on $ORAC_{FL}$ and PCL. The $ORAC_{FL}$ and PCL values for all products (frozen and thermally processed) throughout 6 months of storage are listed in **Table 3**. Storage did not significantly affect the $ORAC_{FL}$ values of IQF, canned-in-syrup blended, canned-in-water blended, clarified juice, nonclarified juice, or puree. For most products (IQF, canned-in-syrup blended, canned-in-water blended, and nonclarified juice), no changes were observed in PCL values; however, there were some differences observed in clarified juice and purees. The PCL values changed only in clarified juice after 1 month, in which a ~20% decrease from 1 day was observed, with a slight increase at 6 months (**Table 3**). The puree PCL values also decreased at 1 month (with a ~8% loss compared to 1 day), but no subsequent changes were observed. We suspect that the greater antioxidant capacity of polymers formed during storage compensated for the losses of monomeric ACYs, resulting in minimal changes in antioxidant capacity during storage.

Both $ORAC_{FL}$ and PCL were evaluated to provide a more comprehensive evaluation of antioxidant activity. The $ORAC_{FL}$ assay was utilized to evaluate the ability of the extract to scavenge peroxyl radicals, and the PCL assay evaluated the ability to scavenge superoxide radicals. These assays demonstrated very similar responses to processing and storage. Thus, it appears that the peroxyl and superoxide radical scavenging capacity is altered to the same extent by processing and storage.

Table 3. $ORAC_{FL}$ and PCL Values of IQF, Blended Canned-in-Syrup, Blended Canned-in-Water, Clarified Juice, Nonclarified Juice, and Puree As Affected by Storage Time[a]

product	storage time	$ORAC_{FL}$ (μmol of TE/g of berry)	PCL (μmol of TE/g of berry)
IQF	1 day	97.2 ± 10.1a	19.5 ± 1.6a
IQF	1 month	84.9 ± 2.1a	19.9 ± 1.1a
IQF	3 months	84.5 ± 6.9a	20.4 ± 1.5a
IQF	6 months	96.7 ± 3.2a	22.4 ± 0.6a
canned-in-syrup	1 day	75.8 ± 2.2a	15.2 ± 0.8a
canned-in-syrup	1 month	72.3 ± 3.6a	14.6 ± 1.3a
canned-in-syrup	3 months	74.9 ± 3.0a	14.9 ± 0.7a
canned-in-syrup	6 months	71.1 ± 5.7a	14.3 ± 1.2a
canned-in-water	1 day	71.1 ± 1.5a	16.5 ± 1.1a
canned-in-water	1 month	81.3 ± 2.2a	15.6 ± 0.5a
canned-in-water	3 months	68.3 ± 7.0a	14.9 ± 0.6a
canned-in-water	6 months	72.8 ± 5.2a	17.3 ± 1.1a
clarified juice	1 day	43.8 ± 0.8a	10.1 ± 0.2a
clarified juice	1 month	42.6 ± 3.0a	8.1 ± 0.3bc
clarified juice	3 months	48.0 ± 2.3a	7.6 ± 0.3c
clarified juice	6 months	47.7 ± 0.4a	8.7 ± 0.3b
nonclarified juice	1 day	43.0 ± 0.8a	9.4 ± 1.2a
nonclarified juice	1 month	42.1 ± 2.3a	10.3 ± 0.7a
nonclarified juice	3 months	41.5 ± 2.0a	9.0 ± 0.8a
nonclarified juice	6 months	45.5 ± 2.5a	9.5 ± 0.6a
puree	1 day	85.0 ± 4.6a	19.0 ± 0.7a
puree	1 month	79.1 ± 4.1a	17.4 ± 0.6ab
puree	3 months	74.5 ± 7.4a	15.5 ± 1.3b
puree	6 months	85.6 ± 5.7a	14.8 ± 0.9b

[a] Values represent means ± standard error ($n = 3$ for $ORAC_{FL}$; $n = 6$ for PCL). Means within columns and treatment group with different letters are significantly different ($p \leq 0.05$).

Monomeric ACYs, $ORAC_{FL}$, and PCL in Canned Berries and Brine. Most canned berries are consumed after the brine has been drained from the product; therefore, in addition to evaluating changes in the whole can (as blended cans), we included analyses of berries and brine individually. The monomeric ACY, $ORAC_{FL}$, and PCL values of berries and brine for canned-in-syrup and canned-in-water products are shown as total levels in each can (**Figure 6**). As observed with the blended canned products (**Figure 3**), the total monomeric ACYs decreased with processing and storage; however, losses were observed not only in the berries but in the brine as well. Overall, the ratio of berry/brine monomeric ACYs decreased from 3.7 to 2.0 over 6 months of storage for canned-in-syrup products and decreased from 3.8 to 2.3 over 3 months of storage, with an increase to 2.9 at 6 months of storage, for canned-in-water products. Thus, although both berries and brines were subject to losses in monomeric ACYs, the greatest losses occurred in the berries. Consistent with our findings, significant losses of ACYs occurred during storage of canned plums (46% loss over 47 days at 30 °C) (*34*), and canned cherries (42% loss over 5 months at 22 °C) (*31*), with approximately 50% of the ACYs migrating from the fruit into the syrup. Both of these studies reported that ACY losses were ameliorated by refrigerated storage.

For canned-in-syrup and canned-in-water berries, significant decreases in $ORAC_{FL}$ and PCL values were observed; however, losses in the berries were often partly compensated by increases in the brine. Although in blended cans there were no differences observed over storage, there were losses in total $ORAC_{FL}$ (in the summed berries and brine) for canned-in-syrup after 1 month

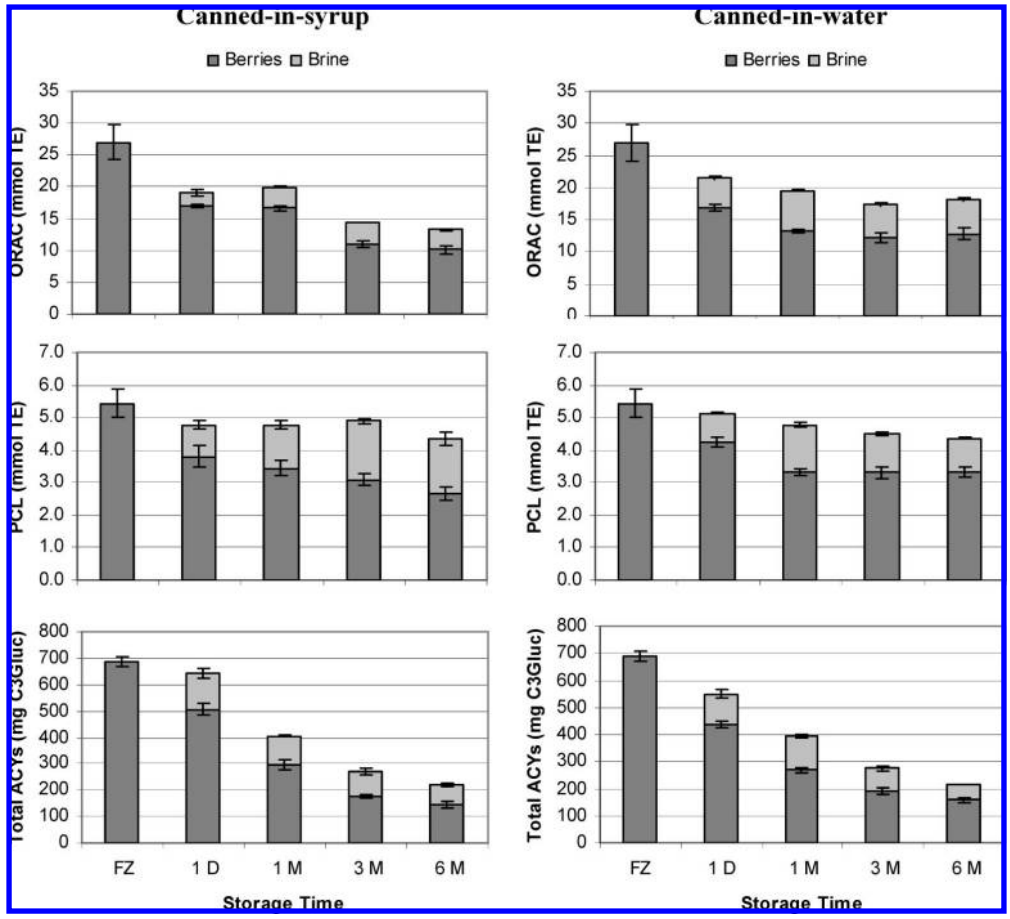

Figure 6. $ORAC_{FL}$, PCL, and total monomeric anthocyanin values in berry and brine fractions of the canned products [canned-in-syrup (CS) and canned-in-water (CW)]. Bars represent standard error of the mean ($n = 6$ for PCL and monomeric ACYs; $n = 3$ for $ORAC_{FL}$).

and canned-in-water after 1 day, although subsequent changes were minimal. With PCL (for berries and brine summed), there was a slight decrease in canned-in-syrup after 3 months and in canned-in-water after 1 month. Again the changes were minimal thereafter.

Losses in monomeric ACYs, $ORAC_{FL}$, and PCL with processing are problematic, because blackberries, like most berries, are readily consumed in processed forms. This is a significant concern in all processed products, but particularly juice products, in which up to 55% of the original anthocyanins were lost. Significant amounts of antioxidant-rich monomeric ACYs and polymers were retained in the presscake, indicating that blackberry juice byproducts are a potential source of nutraceutical ingredients. In canned products significant quantities of anthocyanins leached out of the berries into the brine (between 21 and 33%) during processing and storage. Hence, consumers will not receive the same levels of anthocyanins with the exclusion of brine or syrup from the canned product, although they should be aware that consumption of the syrup will increase caloric intake.

The significant increase in polymeric color with storage with the corresponding loss in monomeric ACYs resulted in unchanged total antioxidant capacity, suggesting that by evaluation with in vitro antioxidant techniques (i.e., $ORAC_{FL}$ and PCL) that the shelf-stable products possibly retain health benefits over long-term storage. However, this conclusion may not be valid when the molecular structure of the polymers is considered, because the possibility of absorption of high molecular mass (>1500 Da) compounds is not likely, based on other studies involving hydrolyzable and condensed tannins (*36, 37*). Further research is needed to characterize the polymers that form in response to processing and storage and to determine their bioavailability in vivo.

ABBREVIATIONS USED

ACY, anthocyanin; AUC, area under the curve; HPLC, high-performance liquid chromatography; IQF, individually quick frozen; ORAC, oxygen radical absorbing capacity; PCL, photochemiluminescence; TE, Trolox equivalents.

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