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

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ABSTRACT: This study evaluated the effects of processing and 6 mo of storage on total monomeric anthocyanins, percent polymeric color, and antioxidant capacity of blueberries that were canned in syrup (CS), canned in water (CW), pureed, and juiced (clarified and nonclarified). Total monomeric anthocyanins, percent polymeric color, and oxygen radical absorbing capacity (ORAC) assay using fluorescein ($ORAC_{FL}$) were determined postprocessing after 1 d, and 1, 3, and 6 mo of storage. Thermal processing resulted in marked losses in total anthocyanins (28% to 59%) and $ORAC_{FL}$ values (43% to 71%) in all products, with the greatest losses occurring in clarified juices and the least in nonclarified juices. Storage at 25 °C for 6 mo resulted in dramatic losses in total anthocyanins, ranging from 62% in berries CW to 85% in clarified juices. This coincided with marked increases in percent polymeric color values of these products over the 6-mo storage. The $ORAC_{FL}$ values showed little change during storage, indicating that the formation of polymers compensated for the loss of antioxidant capacity due to anthocyanin degradation. Methods are needed to retain anthocyanins in thermally processed blueberries.

Keywords: anthocyanins, blueberries, $ORAC_{FL}$, processing, storage

Introduction

Berries, especially blueberries (*Vaccinium corymbosum* L.), have received much attention due to their positive role in human health and disease prevention. The protective effects of blueberries against chronic diseases have generally been attributed to the wide array of polyphenolics such as anthocyanins, flavan-3-ols, proanthocyanidins, and flavonols present in the fruit (Prior and others 2001; Cho and others 2004), which are responsible for the high free radical scavenging capacity of blueberries measured by several *in vitro* assays (Moyer and others 2002; Pellegrini and others 2003; Wu and others 2004). The abundance of antioxidants in blueberries is thought to protect against oxidative stress initiated by reactive oxygen species, in addition to numerous other health-promoting properties (Hou 2003; Zafra-Stone and others 2007).

Blueberries, like other berries, are not only available fresh but also are available for consumption in several thermally processed forms (jellies, jams, juices, canned, and purees). Several studies have investigated the effects of processing on berry anthocyanins. Freezing and subsequent frozen storage have been shown to have minimal effects 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. Kalt and others (2000) reported that various commercial lowbush blueberry products varied significantly in antioxidant capacity, but the source of fruit, product formulations, and processing techniques were unknown. In another study involving commercial blueberry products, the researchers reported that semipurified extracts obtained from thermally processed products retained most of the antioxidant activity and total phenolics found in unprocessed whole fruit, but the antiproliferation activities of thermally processed products were lacking or greatly diminished (Schmidt and others 2005). Information is limited on how different processing methods and long-term storage affect the nutritional quality of blueberries prepared from the same raw material. 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 blueberries.

Materials and Methods

Blueberry samples

Blueberries (cv. Bluecrop) harvested at the fully ripe stage were obtained from a commercial grower in Fayetteville, Ark., U.S.A., in

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June 2005. The fruit was stored at –20 °C for less than 1 mo prior to processing.

Juice processing

The diagrammatic scheme of juice processing with sampling points indicated (1 to 8) 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 a temperature of 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 XXL® (Novozyme, Bagsvaerd, Denmark) and incubating the mash for 1 h at 40 °C. 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, 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., Erie, Pa., U.S.A.) 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 (CW) and Canned-in-syrup (CS) processing

Blueberries were canned by the method of Downing (1996). Frozen berries (278 g) were added to 303 × 406 (3 and 3/16 inches in diameter and 4 and 6/16 inches in height) cans. Syrup was prepared by adding Sweetose 4300 corn syrup (Tate and Lyle, London, U.K.) to boiling water to reach a final reading of 40 °Brix. Boiling syrup (for CS cans) or water (for CW cans) were added to the cans to the brim and cans were exhausted for 4 min in a steam box (American Sterilizer Co.) at 87.8 to 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 draining brine from the berries through a number 8 sieve screen for 3 min.

Puree processing

Frozen berries were allowed to thaw and homogenized for 1 min on high speed using a commercial food processor. Blended berries were immediately added to the 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,

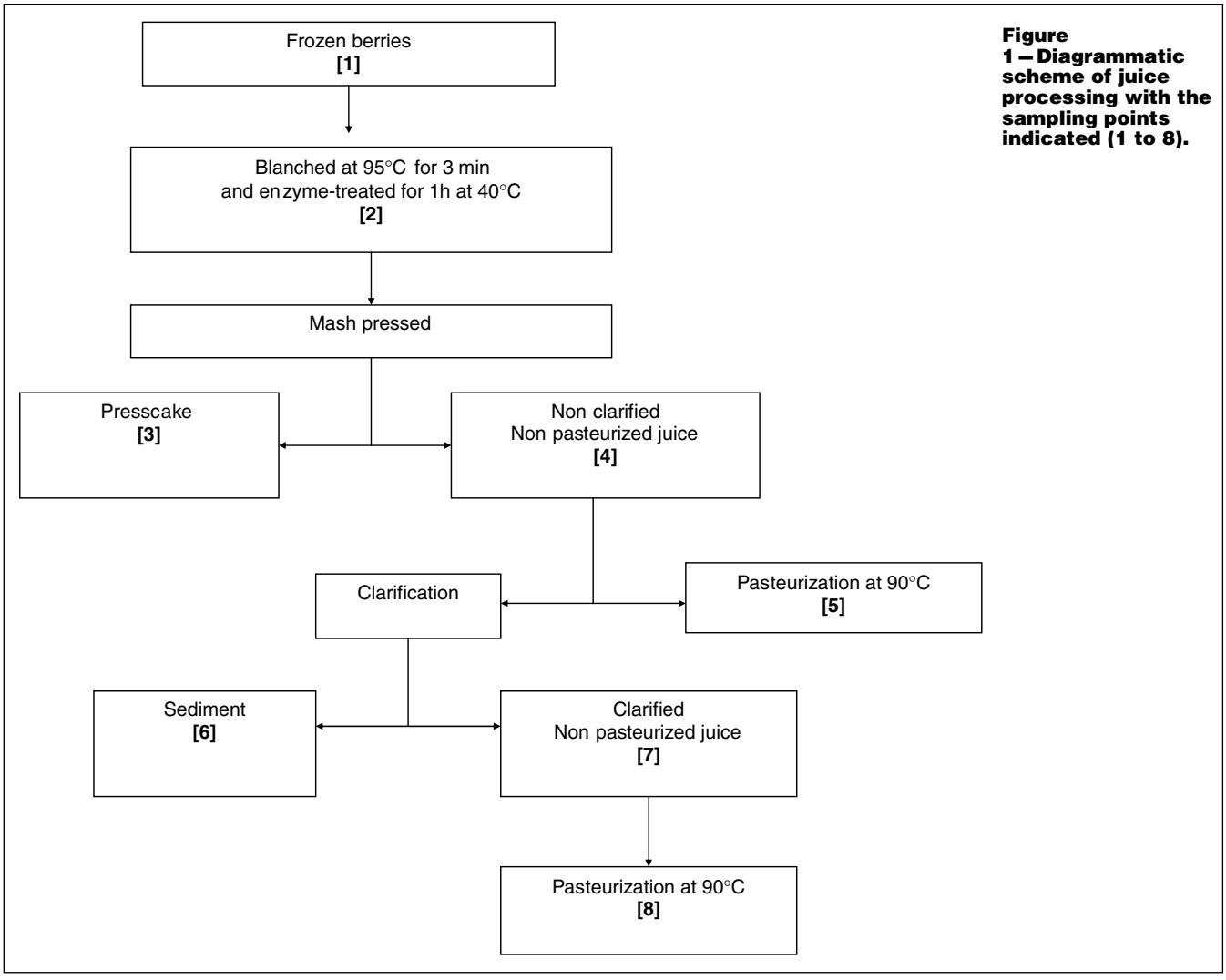


Figure 1—Diagrammatic scheme of juice processing with the sampling points indicated (1 to 8).

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 the extraction of anthocyanins, 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, juice, water, and syrup fractions from canned 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 1.0 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 (Waters Corp.). Individual anthocyanin monoglycosides and acylated anthocyanin derivatives were quantified as delphinidin, cyanidin, petunidin, peonidin, and malvidin glucoside equivalents using external calibration curves (ranging from 5 to 125 μg/mL) of a mixture of anthocyanin glucosides (delphinidin, cyanidin, petunidin, peonidin, and malvidin) obtained from Polyphenols Laboratories AS (Sandnes, Norway). Total anthocyanins were calculated as the sum of individual anthocyanin monoglycosides and acylated anthocyanin derivatives, with 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 (2005). 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, Calif., U.S.A.). For analysis, 0.2 mL of 0.90 M potassium metabisulfite were added to 2.8 mL diluted sample (bisulfite bleached sample) and 0.2 mL of DI water were 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:

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

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

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

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 7.0) 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 original berry.

Calculations

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

$$C_{product} * R = C_{berry}$$

where $C_{product}$ = 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.

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

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

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

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

This calculation allowed for canning media (syrup and water) and berry distribution with processing and storage to be determined.

Statistical analysis

All data were reported as means ± standard error of the 5 samples taken from each processed product at each sampling time. 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 SB–author request means were determined by Student's t-test.

Results and Discussion

Processing and storage effects on monomeric anthocyanins and polymeric color

Juices. Changes in total monomeric anthocyanins were evaluated during different steps in juice processing (Table 1). The blanching treatment did not affect anthocyanin levels in the berries, but 15% of the original concentration of anthocyanins was lost to the presscake during juice pressing, and 25% of the original concentration of anthocyanins was removed as sediment in the juice clarification step. Previous studies reported that 18% (Skrede and others 2000) and 42% to 55% (Lee and others 2002)

of the anthocyanins were lost to the presscake during blueberry juice processing, and 8% was removed during juice clarification (Lee and others 2002). Prior to pasteurization, 80% of the original monomeric anthocyanins were retained in nonclarified juices, indicating that approximately 5% of the anthocyanins were degraded during juice processing operations. Other studies report much lower retention of anthocyanins (22% to 34%) in nonclarified pressed juices (Skrede and others 2000; Lee and others 2002; Srivastava and others 2007). The marked differences in anthocyanin retention among our results and others may be attributed to differences in thawing procedures and blanch treatments. In our study, frozen berries were thawed and quickly blanched in a steam kettle, while in previous studies, fruit were thawed for 12 h at 5 °C, followed by water (Srivastava and others 2007) and steam (Lee and others 2002), and blanching (Srivastava and others 2007) and no blanch treatment (Skrede and others 2000). We suspect that the rapid inactivation of polyphenoloxidase (PPO) by the steam treatment employed in our study resulted in much higher retention of anthocyanins. In contrast to nonclarified juice, only 46% of the original monomeric anthocyanins were retained in clarified juices, indicating that approximately 14% of the anthocyanins were degraded during juice processing operations. Lee and others (2002) reported that less than 20% of anthocyanins were retained in clarified juices and found that only 8% of the anthocyanins were lost during the clarification step, as opposed to 25% loss during clarification in our study. The greater loss observed in our study may be due to use of a standard centrifuge, whereas Lee and others (2002) used a continuous centrifuge separator. Pasteurization of nonclarified and clarified juices resulted in minor monomeric anthocyanin losses of 8% and 5%, respectively. Based upon these results and those of Skrede and others (2000), who reported a 4% increase in anthocyanins following pasteurization, it appears that anthocyanins are well retained during pasteurization. The losses of monomeric anthocyanins in our study were most likely due to the formation of anthocyanin polymers during the juice processing steps. The presscake and sediment had polymeric color values of 30% and 12%, respectively, indicating that monomeric anthocyanins were extensively polymerized, presumably, during the depectinization step that was performed at 40 °C for 1 h. Polymerization could be due to endogenous enzymes in blueberries that were not totally inactivated by the blanching treatment. According to Skrede and others (2000), the addition of a blanched blueberry-pulp extract to blueberry juice resulted in no degradation of anthocyanins, whereas addition of an unblanched extract caused a 50% loss of anthocyanins, suggesting an enzymatic role in anthocyanin degradation.

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

Processing step	Total monomeric anthocyanins (mg/100 g berry)	% Polymeric color	$ORAC_{FL}$ (μmol TE/g berry)
Fresh (1)	166.7 ± 1.1a	0.6 ± 0.16c	102.4 ± 0.3a
Blanched (2)	166.5 ± 3.8a	8.6 ± 0.4bc	76.2 ± 2.6b
Presscake (3)	25.2 ± 1.6e	30.1 ± 7.0a	17.3 ± 0.3f
Juice, NC, NP (4)	133.7 ± 5.7b	8.6 ± 1.2bc	50.4 ± 0.4d
Juice, NC, P (5)	120.8 ± 10.5b	10.2 ± 0.7b	57.8 ± 1.9c
Sediment (6)	41.4 ± 5.0d	12.1 ± 2.4b	20.3 ± 0.6f
Juice, C, NP (7)	76.8 ± 2.7c	6.2 ± 1.2bc	28.1 ± 0.7e
Juice, C, P (8)	68.6 ± 1.1c	7.5 ± 1.4bc	29.3 ± 0.8e

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

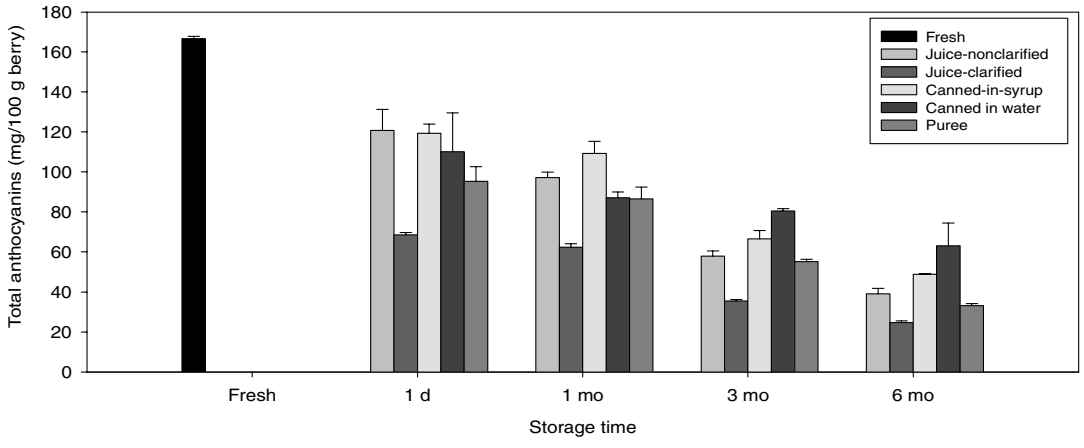
Both peroxidase (POD) and PPO have been shown to cause anthocyanin degradation in the presence of cofactors such as chlorogenic acid for PPO (Kader and others 1997a, 1997b) and chlorogenic acid and H_2O_2 for POD (Kader and others 2002). Another potential mechanism for polymerization involves condensation 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). The degradation of anthocyanins prior to pasteurization could also be the result of side activities of the Pectinex Smash XXL enzyme preparation used for depectinization. However, no aglycones of anthocyanins were observed in HPLC chromatograms of samples following enzyme treatment, indicating that the enzyme preparation did not contain sufficient glycosidase activity to produce aglycones.

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

Compared to pasteurized juice, total monomeric anthocyanins in nonclarified juices decreased linearly during storage with losses of 20%, 52%, and 68% observed over 1-, 3-, and 6-mo storage, respectively. Similar losses of 9%, 48%, and 64% were observed for clarified juices over 1-, 3-, and 6-mo storage, respectively. After 6-mo storage, nonclarified and clarified juices contained only 23% and 15% of the anthocyanins present in fresh berries. Consistent with our results, Srivastava and others (2007) reported that only 50% of the anthocyanins were retained in blueberry juices stored for 60 d at 23 °C. Similar to other thermally processed products, anthocyanin losses during storage were accompanied by linear increases in percent polymeric color values, which increased from 10% to 25% in nonclarified juices and from 8.0% to 17% in clarified juices over the 6-mo storage.

Canned. Processing of berries CS or CW resulted in total monomeric anthocyanin losses of 28% and 34%, respectively, compared to the original levels found in fresh berries when the entire contents of the can were blended and analyzed. Percent polymeric color values increased from 1% (fresh fruit) to 22% and 7% in berries CS and berries CW, respectively, in response to processing. Levels of monomeric anthocyanins continued to decline in a linear fashion during storage, with losses of 35%, 60%, and 71% observed in berries CS stored 1, 3 and 6 mo, respectively. Comparable monomeric anthocyanin losses of 48%, 52%, and 62% were observed in berries CW over 1-, 3-, and 6-mo storage, respectively. Anthocyanins were extensively polymerized during storage, with percent polymeric color values increasing from 1% (fresh) to 11%, 13%, and 18% in berries CW over 1-, 3-, and 6-mo storage, respectively. After the large increase in response to processing, the percent polymeric color values remained relatively constant (22% to 26%) over 6-mo storage in berries CS. 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, Chaovanalikit and Wrolstad (2004) found that anthocyanins in Bing cherries increased slightly after canning, but levels declined 38% over a 5-mo storage at 22 °C. 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 CS 14% to 23% of monomeric anthocyanins diffused out of the fruit into the aqueous medium over the 6 mo storage, while 77% to 86% of the monomeric anthocyanins were retained in the berries (Figure 3). In berries CW, 17% to 23% of the monomeric anthocyanins diffused out of the fruit into the syrup, while 77% to 83% were retained in the berries over the 6-mo storage (Figure 4). Leaching of anthocyanins in blueberries in response to canning and storage was less than previously reported values for canned Bing cherries (50% diffusion; Chaovanalikit and Wrolstad 2004) and strawberries (60% diffusion; Ngo and others



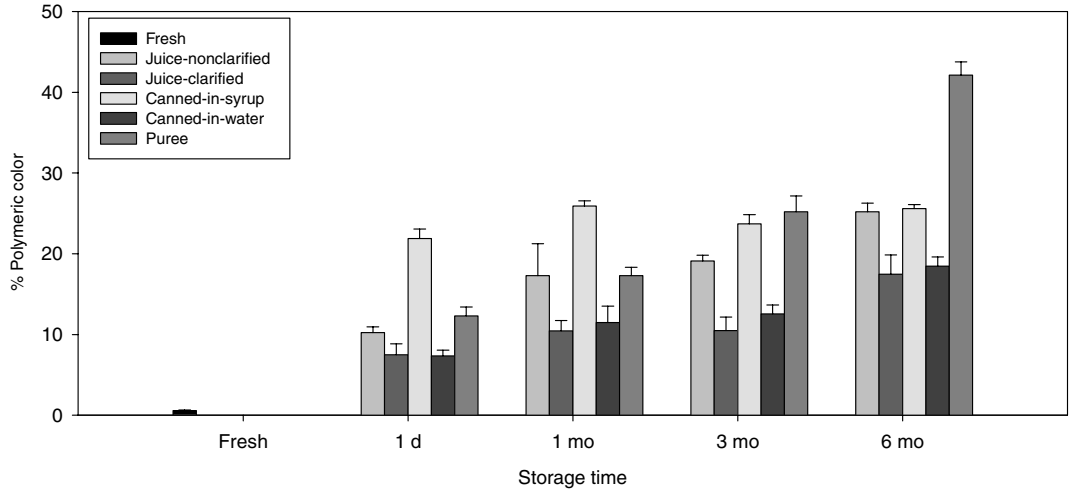


Figure 2—Total momomeric anthocyanins and percent polymeric color of thermally processed blueberry products as affected by processing and storage at 25 °C. Bars represent ± SEM (n = 5).

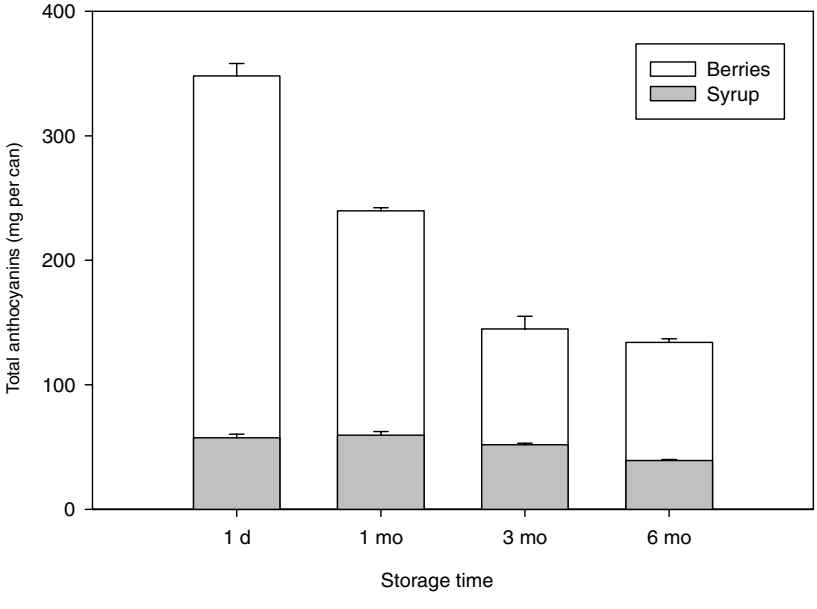


Figure 3—Total monomeric anthocyanins in berry and syrup fractions isolated from canned blueberries as affected by storage at 25 °C. Bars represent ± SEM (n = 5).

2007). Since the bloom layer of blueberries composed of epicuticular waxes is reported to be destroyed by cooking (Sapers and others 1984), we suspect that the cuticle prevented extensive leaching of anthocyanins into the liquid canning medium.

Puree. Processing of puree resulted in a 43% loss in total monomeric anthocyanins, compared to original levels found in fresh fruit, while polymeric color values increased from 1% to 12%. Levels of total monomeric anthocyanins continued to decrease during room temperature storage with losses of 48%, 67%, and 80% observed after 1-, 3-, and 6-mo storage, respectively. The losses of total monomeric anthocyanins were accompanied by increased polymeric color values, which increased from 1% (fresh fruit) to 17%, 25%, and 42% after 1-, 3-, and 6-mo storage, respectively. 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).

The large increase in polymeric color values and corresponding loss of monomeric anthocyanins with storage in all thermally processed products may be due to several factors described previously, including residual enzyme activity or condensation reactions of anthocyanins with other phenolics.

Processing and storage effects on $ORAC_{FL}$

The effects of processing and storage on the hydrophilic antioxidant capacity of blueberry products were evaluated by the $ORAC_{FL}$ assay, which measures the ability of extracts to scavenge peroxyl radicals (Figure 5). The $ORAC_{FL}$ value of fresh Bluecrop blueberries (102.4 μmol TE/g) was much higher than the previously reported value of 51.8 μmol TE/g (Cho and others 2004). This discrepancy may be explained by the growing season effect described by Howard and others (2003).

Juices. Changes in antioxidant capacity were evaluated during different steps in juice processing (Table 1). The blanching treatment resulted in a 26% loss of antioxidant capacity, while 17% and 20% of the antioxidant capacity was lost to the presscake and sediment, respectively. Prior to pasteurization only 49% and 28% of the original antioxidant capacities were retained in nonclarified

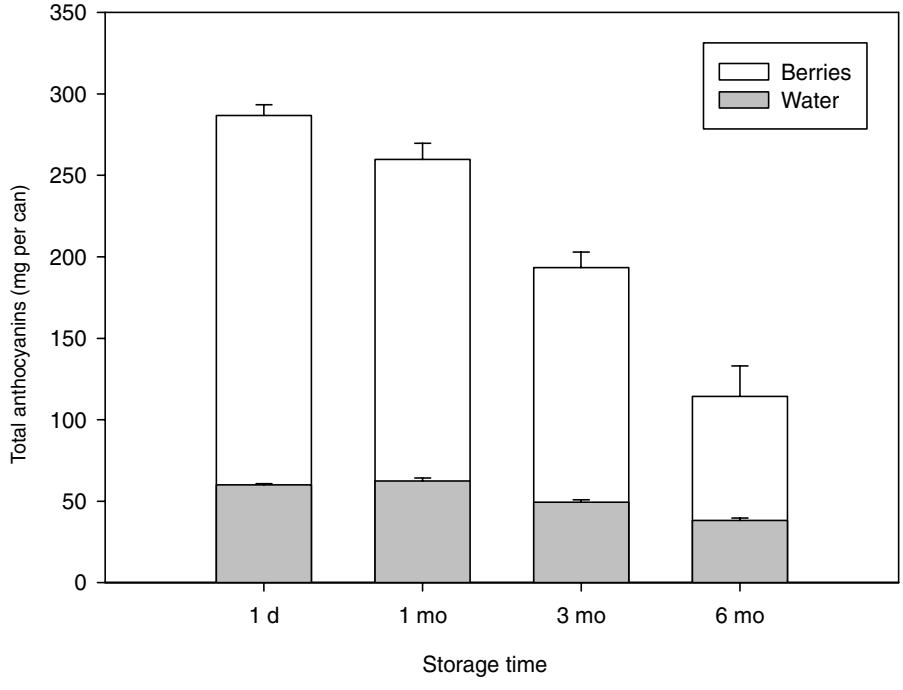


Figure 4—Total monomeric anthocyanins in berry and water fractions isolated from canned blueberries as affected by storage at 25 °C. Bars represent ± SEM (n = 5).

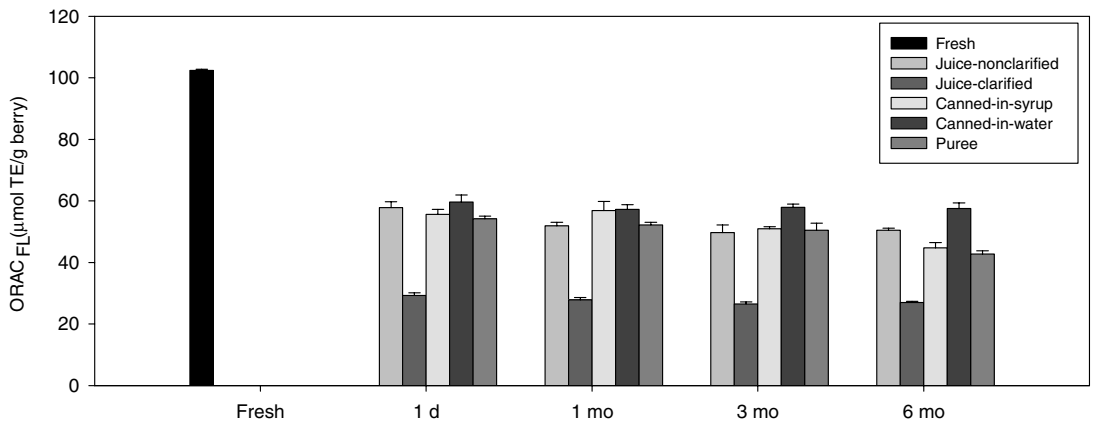


Figure 5—$ORAC_{FL}$ of thermally processed blueberry products as affected by processing and storage at 25 °C. Bars represent ± SEM (n = 5).

and clarified juices, respectively. The losses in antioxidant capacity prior to pasteurization were consistent with the marked losses of monomeric anthocyanins observed in nonclarified (20%) and clarified (54%) juices. Despite minor losses in monomeric anthocyanins (8% in nonclarified and 5% in clarified) due to pasteurization, the antioxidant values of nonclarified and clarified juices increased 7% and 1% after 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), or the formation of anthocyanin polymers. The antioxidant capacity values of nonclarified and clarified juices remained stable over the 6-mo storage, despite marked losses of total anthocyanins (Figure 5). As observed in the other thermally processed products, the antioxidant capacity of polymeric anthocyanins formed during storage likely compensated for the loss of antioxidant capacity as a result of monomeric anthocyanin degradation.

Canned. The antioxidant capacity of berries CS and berries CS decreased by 46% and 42% in response to processing, reflecting the 28% and 34% losses of monomeric anthocyanins in the products (Figure 5). Despite marked losses of anthocyanins during storage, the antioxidant capacity values remained relatively stable, although a 12% loss occurred from 1- to 6-mo storage in berries CS. We suspect that the antioxidant activity of anthocyanin polymers formed during storage compensated for the loss of monomeric anthocyanins (Tsai and Huang 2004; Tsai and others 2004).

Puree. Processing resulted in a 47% loss in antioxidant capacity, which was consistent with the 43% loss of monomeric anthocyanins (Figure 5). However, despite significant losses of monomeric anthocyanins during storage the $ORAC_{FL}$ values remained relatively stable during storage, although a 9% loss was observed from the 1- to 6-mo storage. Similar to results obtained for juices and canned berries, the retention of antioxidant capacity during storage most likely reflected antioxidant contribution by anthocyanin polymers that increased during storage.

Conclusions

Processing blueberries into various forms resulted in significant losses of monomeric anthocyanins (28% to 59%) and antioxidant capacity (53% to 71%), which most likely was due to enzymatic polymerization and/or degradation of anthocyanins prior to pasteurization or polymerization reactions with anthocyanins and other phenolic compounds. Monomeric anthocyanins were extensively degraded during storage in all thermally processed products (canned, juices, and purees), with less than 40% of the original total anthocyanins present in the processed products after 6 mo. In canned products, significant amounts of monomeric anthocyanins (14% to 25%) 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 studies are needed to identify the anthocyanin polymers and to determine their bioavailability *in vivo.*

Acknowledgments

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