JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Processing and Storage Effects on Procyanidin Composition and Concentration of Processed Blueberry Products

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Blueberries are a rich source of procyanidins that may contribute to the reduced risk of chronic disease; however, because of seasonal availability, the berries are commonly consumed in thermally processed forms after long-term storage. In this study, we evaluated the effects of processing and 6 months of storage on procyanidin composition and content of blueberries that were canned in syrup (CS), canned in water (CW), puréed, and juiced (nonclarified and clarified). Processing blueberries into various forms resulted in significant losses of total procyanidins, with only 19 and 23% being retained in nonclarified and clarified juices, 41% retained in purées, and 65 and 78% being retained in berries CS and CW. The mono- and dimers were retained to a much greater extent than larger oligomers in all products following processing. Procyanidins were further degraded during 6 months of storage, with only 8% and 11% retained in clarified and nonclarified juices, 7% retained in puréed, and 22 and 32% retained in berries CS and CW. Similar to results obtained following processing, mono- and dimers were better retained than larger oligomers. Methods are needed to prevent procyanidin losses during processing and storage.

KEYWORDS: Blueberries; procyanidins; processing; storage

INTRODUCTION

Blueberries are a rich source of different types of polyphenolics, including anthocyanins, flavonols, hydroxycinnamic acids, flavan-3-ols, and procyanidins (*1*−*3*). The polyphenolics present in blueberries as well as other fruits and vegetables are thought to play an important role in health promotion through a variety of physiological activities, such as antioxidant, anti-inflammatory, antihypertensive, and antiallergy properties, in addition to their ability to regulate enzyme activities, gene regulation, and cell-signaling pathways (reviewed in refs *4* and *5*).

Flavan-3-ols and procyanidins have received much attention because of their antioxidant capacity, free-radical-scavenging ability, and cardio-protective effects (*6*). The most common procyanidins are mixtures of oligomers and polymers consisting of (+)-catechin and/or (−)-epicatechin units linked mainly through C4 → B8 bonds (B type). Blueberries contain appreciable levels of procyanidins compared to other fruits and vegetables (*3*). Blueberry procyanidins have an average degree of polymerization (DP) of 15.9, in large part because of the polymers that range in DP from 19.9 to 114.1, with epicatechin and catechin accounting for 91 and 2.6% of the extension units, respectively (*7*).

Blueberries are commonly preserved by thermal processing methods, including juicing, canning, and puréeing. The different thermal processing methods have been shown to reduce polyphenolic levels in blueberries (*8*−*11*), but information is lacking on how different thermal processing methods and storage of processed products influence the procyanidin composition and content of blueberries. Studies on other processed fruits, including canned peaches (*12*, *13*), apple purée (*14*), and juices (*15*), indicate that substantial losses of procyanidins can occur in response to processing and storage. It is important to measure changes in both procyanidin composition and content, because bioavailability of procyanidins varies according to the degree of polymerization. According to Deprez et al. (*16*), procyanidin mono-, di-, and trimers can be absorbed, while higher oligomers and polymers are not absorbed. Moreover, only mono-, di-, and trimers have been detected in plasma and urine following consumption of procyanidin-rich diets (*17*−*20*). Hence, losses of procyanidin mono-, di-, and trimers could have an adverse effect on the health-promoting properties of blueberries.

This study evaluated changes in procyanidin composition and content in response to thermal processing and storage of juiced (nonclarified and clarified), canned in water (CW), canned in syrup (CS), and puréed blueberries.

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10.1021/jf803015s CCC: $40.75
Published on Web 02/12/2009

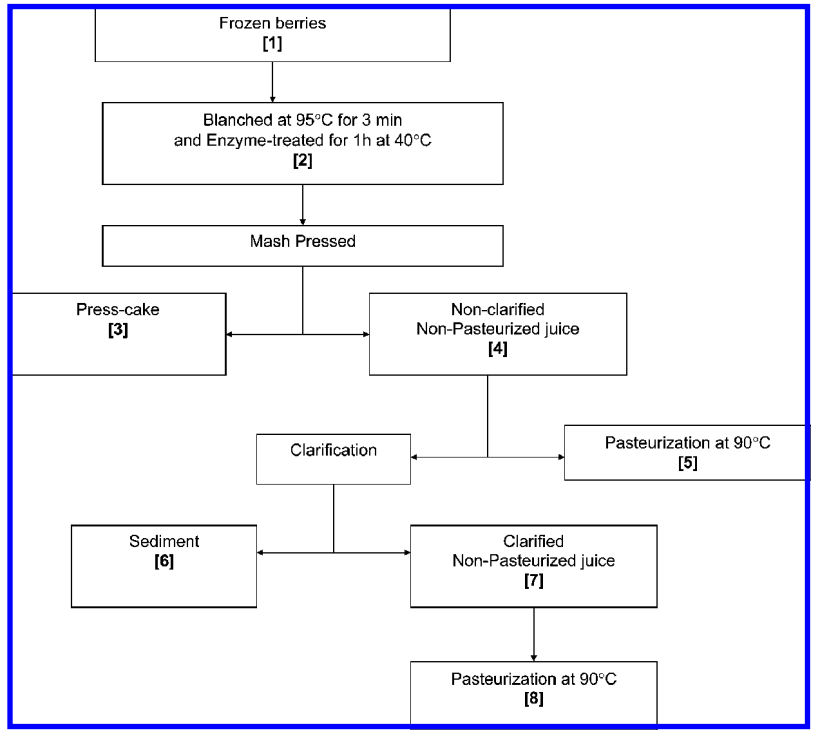

Figure 1. Process for blueberry juice production indicating samples (1−8) that were collected for analysis.

MATERIALS AND METHODS

Materials. Blueberries (cv. Bluecrop) harvested at the fully ripe stage were obtained from a commercial grower in Fayetteville, AR, in June 2005. The fruit were stored at −20 °C for less than 1 month prior to processing.

Processing. Blueberries were processed into nonclarified and clarified juices, puréed, and canned products (water or syrup), as previously described (*11*). The diagrammatic scheme of juice processing with sampling points indicated (1−8) is shown in **Figure 1**.

Extraction and Purification of Procyanidins. Prior to extraction, the entire contents of the canned samples (berries + syrup or berries + water) were homogenized for 1 min at high speed using a commercial food processor. Blended samples (5 g FW) of canned products or purées and 5 mL of juices were homogenized with 20 mL of acetone/water/acetic acid (70:29.5:0.5, 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 with 10 mL of extraction solvent. The filtrates were adjusted to a final volume of 50 mL with extraction solvent. Samples were further purified by solid-phase extraction using Sephadex LH-20. After equilibrating 3 g (DW) of Sephadex LH-20 with 30% methanol overnight, the hydrated material was manually packed into 6 × 1.5 cm columns. Samples were prepared for further purification by evaporating 40 mL of the acetone/water/acetic acid (70:29.5:0.5, v/v/v) extract under vacuum in a SpeedVac (SPE 1010, Thermo Savant, Holbrook, NY) at 25 °C to approximately 12 mL. The concentrated extract was then loaded onto the Sephadex LH-20 column, and the column was washed with 40 mL of 30% methanol in water (approximately 3 column volumes) to remove sugars and other phenols. Procyanidins were then eluted by washing the column with 80 mL of 70% acetone in water. The procyanidin fraction was evaporated to dryness using a SpeedVac and reconstituted in 5 mL of extraction solvent (70:29.5:0.5 acetone/water/acetic acid, v/v/v). The reconstituted samples were passed through 0.45 μm PTFE syringe filters (Varian, Inc., Palo Alto, CA) prior to high-performance liquid chromatography (HPLC) analysis.

HPLC Analysis of Procyanidins. The procyanidin analysis by HPLC was performed according to an adapted method of Kelm et al. (*21*) with a 250 × 4.6 mm i.d., 5 μm Develosil diol column (Phenomenex, Torrance, CA). The mobile phase consisted of a binary gradient of acetonitrile/acetic acid (98:2 v/v) (A) and methanol/water/acetic acid (95:3:2, v/v/v) (B). The flow rate was 0.8 mL/min with a linear gradient as follows: 0−35 min, 0−40% B; 35−45 min, 40% B; 45−47 min, 50% B; 47−49 min, 60% B; 49−50 min, 100% B; 50−52 min, 100% B; 53−60 min, 0% B. The column was re-equilibrated with 0% B for 5 min. The procyanidin peaks were monitored by fluorescence detection with excitation at 276 nm and emission at 316 nm using a Waters Model 474 fluorescence detector (Milford, MA). Individual procyanidins with degrees of polymerization (DP) from DP1 to DP8 were quantified using external calibration curves of a mixture of cocoa procyanidin standards obtained from Mars, Inc. (Hackettstown, NJ), with results expressed as milligrams of each individual procyanidin per kilogram of original berry (FW). The HPLC method was recently validated in a multilaboratory study (Robbins et al., in press), where the authors provided detailed information on the performance characteristics for procyanidin standards ranging from mono- to decamer, including calibration ranges, limit of detection (LOD), limit of quantification (LOQ), variation, and resolution. The recovery rates of the individual procyanidins (DP1−DP10) through the extraction and purification procedures were reported previously (*7*). Total procyanidins were calculated as the sum of individual procyanidins, with results expressed as milligrams per kilogram of original berry. Frozen blueberries, blanched blueberries, press cake, and sediment from clarification were analyzed for procyanidin levels, as well as clarified juice (CJ) and nonclarified juice (NCJ), before pasteurization (NP), after pasteurization (P), and after 1, 3, and 6 months of storage.

Calculations. For juices and purées, the procyanidin values were converted to original berry weight using the following calculation:

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

where C_{product} is the concentration of product, R is the ratio of the mass of product produced to the mass of the original berry, and C_{berry} is the 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.

For canned products, the masses (g) of berries and liquid canning media added to the cans were recorded prior to processing. The procyanidin results were corrected for the amount of liquid media added to the cans.

Statistical Analysis. All data were reported as means ± standard error of five samples taken from each processed product at each sampling time. Effects of juice-processing steps on procyanidins were

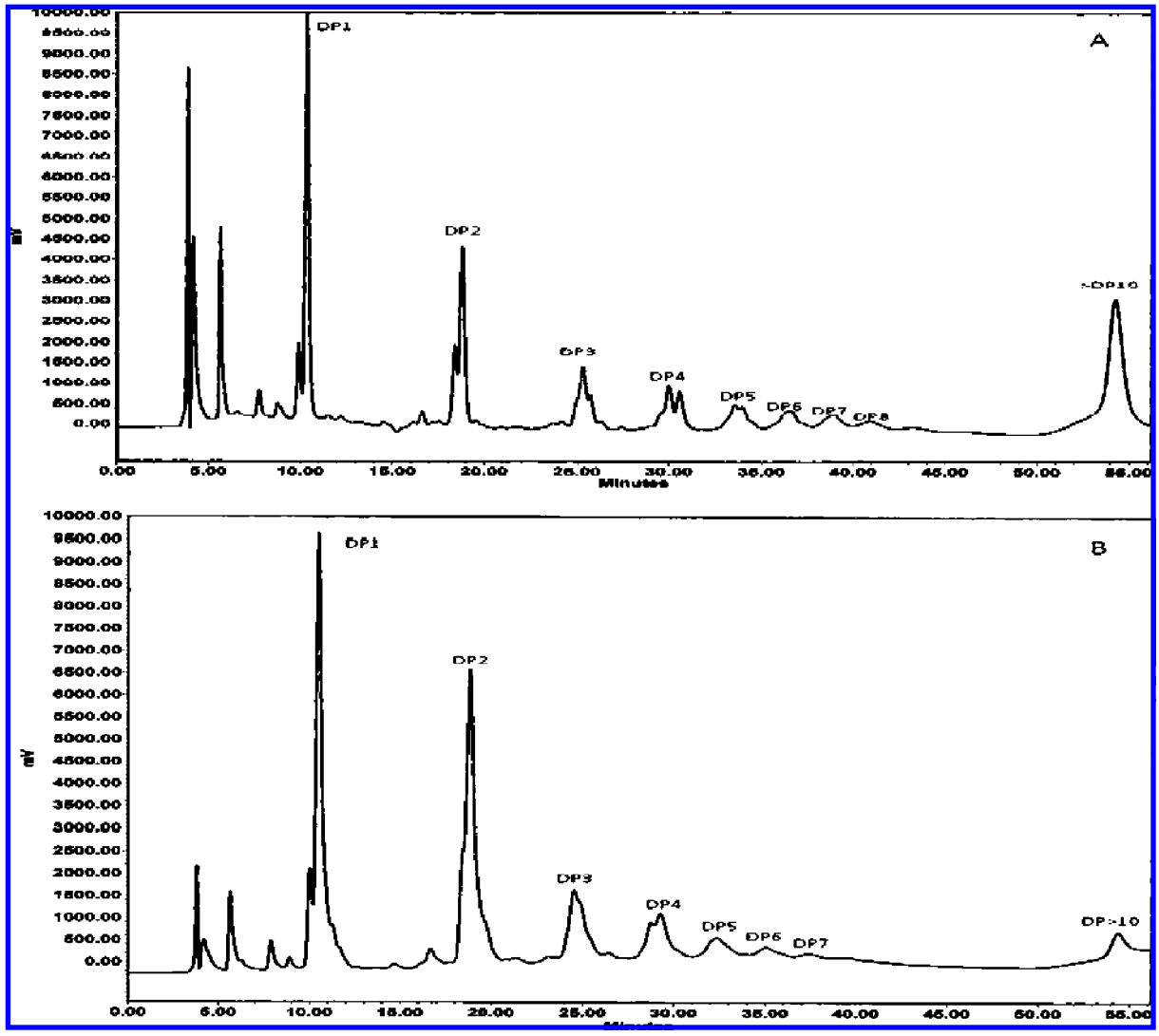

Figure 2. Normal-phase HPLC fluorescence traces of the procyanidins from (**A**) frozen Bluecrop blueberries and (**B**) nonclarified juice 1 day after processing. Labels DP1 (monomer, catechin/epicatechin)−DP8 (octamer) indicate the degree of polymerization of procyanidins.

analyzed by one-way analysis of variance (JMP software, version 6.0, Cary, NC). Significant differences ($p \leq 0.05$) between means were determined by Student's *t* test.

RESULTS AND DISCUSSION

HPLC Analysis of Procyanidins. The HPLC method allowed for efficient separation of procyanidin oligomers in frozen berry extracts from monomers up to octamers (**Figure 2A**). The frozen Bluecrop blueberries contained 33.4, 60.7, 58.4, 60.0, 75.6, 51.4, 16.7, and 22.1 mg/kg FW from monomers to octamers, respectively. These concentrations were similar to the values (mg/kg FW) reported by Gu et al. (*3*) for cultivated highbush blueberries using similar methodology: monomers (40), dimers (72), trimers (54), and tetra- to hexamers (196). A large well-resolved polymer peak representing procyanidins > DP10 was also present in chromatograms of frozen berry extracts. However, the polymer peak was greatly reduced in size and poorly resolved in all extracts of thermally processed samples (**Figure 2B**), making quantification difficult. Hence, only the results for procyanidin oligomers up to DP8 are reported in the paper.

Procyanidin Changes during Processing of Blueberry Juice. The changes in procyanidin composition and content during different steps in juice processing are shown in **Table 1**. The blanching step had a minor effect on levels of mono-, di-, and trimers (>83% retention), but only 64, 59, 34, and 33% of penta-, hexa-, hepta-, and octamers were retained. We suspect that the 138% recovery of dimers following blanching may be the result of enhanced extraction as a result of tissue softening. However, we cannot rule out the possibility that larger oligomers were converted to dimers in response to the thermal treatment. After blanching, 79% of total procyanidins were retained in comparison to levels found in fresh fruit. The greater losses of larger oligomers compared to mono-, di-, and trimers may be attributed to the binding of the large-molecular-weight procyanidins to cell-wall polymers, which occurred once the cells were disrupted following heating and mixing. Procyanidins have been shown to bind readily to cell-wall polysaccharides through hydrogen-bonding and/or hydrophobic interactions (*22, 23*). Presumably, the procyanidin oligomers were bound to the extent that they resisted extraction with the extraction solvent. After juice pressing, 24% of total procyanidins were retained in the press cake, confirming that the compounds have a strong affinity to cell-wall polysaccharides. These results indicate that, in addition to anthocyanins (*8, 9, 11*), blueberry pomace represents a valuable source of procyanidins. Mono- and dimers were well-retained in nonclarified, nonpasteurized juices, but less than 50% of tri- and tetramers, 20% of penta-, hexa-, and heptamers, and 6% of octamers were retained. The nonclarified, nonpasteurized juices retained only 47% of total procyanidins found in fresh

Table 1. Content of Procyanidin Oligomers (mg/kg FW) Throughout Blueberry Juice Processing, with Each Processing Step Corresponding to Steps Indicated in **Figure 1**[a]

	processing step						
procyanidin	[2] blanched	[3] press cake	[4] juice, NC,[b] NP[b]	[5] juice, NC, P[b]	[6] sediment	[7] juice, C,[b] NP	[8] juice, C, P
monomers	27.8 ± 1.0 a (82%)[c]	6.0 ± 0.1 c (18%)	26.3 ± 0.4 a (78%)	13.8 ± 1.0 b (41%)	3.0 ± 0.1 d (9%)	14.1 ± 0.6 b (42%)	12.8 ± 0.4 b (38%)
dimers	83.8 ± 6.9 a (138%)	19.5 ± 0.4 d (32%)	69.3 ± 1.6 b (114%)	29.3 ± 2.5 c (50%)	9.2 ± 0.3 d (15%)	38.5 ± 2.5 c (63%)	35.2 ± 1.1 c (58%)
trimers	53.7 ± 5.0 a (92%)	15.5 ± 0.8 c (26%)	25.9 ± 0.4 b (44%)	12.4 ± 0.7 cd (21%)	5.4 ± 0.3 d (9%)	27.1 ± 1.5 b (47%)	13.8 ± 0.5 cd (24%)
tetramers	46.7 ± 4.6 a (78%)	14.3 ± 0.6 c (24%)	29.9 ± 0.8 b (50%)	12.1 ± 0.9 cd (20%)	5.4 ± 0.2 d (9%)	32.7 ± 4.2 b (54%)	12.2 ± 0.7 cd (20%)
pentamers	48.1 ± 3.5 a (64%)	17.2 ± 0.5 b (23%)	11.9 ± 0.3 bc (16%)	3.6 ± 0.2 d (5%)	6.5 ± 0.3 cd (9%)	15.7 ± 0.5 b (21%)	8.1 ± 0.4 cd (11%)
hexamers	30.5 ± 2.0 a (59%)	11.2 ± 0.4 b (22%)	9.2 ± 0.6 bc (18%)	2.4 ± 0.3 e (3%)	4.2 ± 0.1 d,e (8%)	6.6 ± 0.3 cd (13%)	2.7 ± 0.5 e (5%)
heptamers	5.6 ± 0.3 a (34%)	3.0 ± 0.1 b (18%)	3.4 ± 0.3 b (20%)	0.5 ± 0.2 c (5%)	1.1 ± 0.0 c (7%)	0.9 ± 0.0 c (5%)	0.4 ± 0.1 c (2%)
octamers	7.2 ± 0.6 a (33%)	3.8 ± 0.2 ab (17%)	1.3 ± 0.1 b (6%)	ND	ND	ND	ND
total (monomer−octamer)	296.6 ± 24.3 a (79%)	90.4 ± 2.9 d (24%)	176.0 ± 4.0 b (47%)	70.2 ± 0.2 de (19%)	36.1 ± 1.2 e (10%)	135.5 ± 4.4 c (36%)	85.2 ± 2.5 d (23%)

[a] Values represent means ± standard error ($n = 5$). Means within rows with different letters are significantly different ($p \leq 0.05$). [b] NC, nonclarified; NP, nonpasteurized; P, pasteurized; C, clarified. [c] Values within parentheses represent percent retention compared to frozen berries. Levels of procyanidins (means ± standard error) in frozen berries: monomers (33.7 ± 1.3), dimers (60.7 ± 3.3), trimers (53.7 ± 5.1), tetramers (60.0 ± 3.3), pentamers (75.6 ± 4.0), hexamers (51.4 ± 1.1), heptamers (16.7 ± 1.4), octamers (21.8 ± 3.5), total (monomer−octamer) (378.5 ± 20.7).

Table 2. Content of Procyanidin Oligomers (mg/kg FW) in Nonclarified and Clarified Blueberry Juices over 6 Months of Storage at 25 °C[a]

	storage time		
procyanidin	1 month	3 months	6 months
	Nonclarified Juice		
monomers	12.1 ± 0.8 a (36%)[b]	11.6 ± 0.6 a (34%)	9.5 ± 1.1a (28%)
dimers	27.3 ± 3.3 a (45%)	24.8 ± 1.4 a (41%)	20.0 ± 3.0 a (33%)
trimers	10.0 ± 0.8 a (18%)	9.5 ± 0.5 a (19%)	8.5 ± 1.2 a (15%)
tetramers	9.0 ± 0.8 a (15%)	6.5 ± 0.7 b (11%)	4.7 ± 0.7 b (8%)
pentamers	1.8 ± 0.3 a (2%)	0.7 ± 0.3 b (1%)	ND
total (monomer−pentamer)	59.7 ± 4.6 a (16%)	53.6 ± 2.9 ab (14%)	42.7 ± 5.6 b (11%)
	Clarified Juice		
monomers	11.2 ± 0.8 a (33%)	8.2 ± 1.1 b (24%)	6.3 ± 0.5 b (19%)
dimers	29.7 ± 2.5 a (49%)	22.5 ± 3.0 b (37%)	11.7 ± 1.0 c (19%)
trimers	9.6 ± 0.8 a (18%)	5.9 ± 0.7 b (11%)	4.4 ± 0.4 b (8%)
tetramers	8.2 ± 1.0 a (14%)	5.1 ± 0.4 b (8%)	4.8 ± 0.7 b (8%)
pentamers	4.5 ± 1.5 a (6%)	2.0 ± 0.3 a (3%)	2.9 ± 0.3 a (4%)
total (monomer−pentamer)	61.7 ± 7.5 a (16%)	43.4 ± 4.7 b (11%)	30.3 ± 2.3 b (8%)

[a] Values represent means ± standard error ($n = 5$). Means within rows for each juice type with different letters are significantly different ($p \leq 0.05$). Oligomers > DP5 were nondetectible in the stored juices. [b] Values within parentheses represent percent retention compared to frozen berries.

berries. Pasteurization of nonclarified juices resulted in extensive losses of procyanidins, with only 41, 48, 21, and 20% of mono-, di-, tri-, and tetramers retained, respectively (**Table 1**). Additionally, less than 5% of penta-, hexa-, and heptamers were retained, while no octamers were detected. The nonclarified juices retained only 19% of the total procyanidins present in frozen berries following pasteurization. Our results are consistent with previous studies that found only 25−40% of procyanidins were retained in nonclarified blueberry juice following processing (*9*, *10*).

The juice clarification step also resulted in procyanidin losses, with 10% of the total procyanidins recovered in the sediment following centrifugation. The clarified, nonpasteurized juices retained only 42, 63, 47, and 54% of mono-, di-, tri-, and tetramers, 21% of pentamers, and less than 15% of hexa- and heptamers, while octamers were not detected. Only 36% of total procyanidins were retained in the clarified, nonpasteurized juices. Similar to nonclarified juices, pasteurization of clarified juices resulted in significant losses of procyanidins, with only 38, 58, 24, and 20% retention of mono-, di-, tri-, and tetramers and less than 10% retention of hexa- and heptamers, while octamers were not detected. Only 23% of total procyanidins were retained in the clarified juices following pasteurization. The results indicate that procyanidins were readily lost during juice processing as a result of physical removal (press cake and sediment) and thermal degradation. The mono- and dimers were retained to a much greater extent than the larger oligomers, suggesting that the low-molecular-weight procyanidins bind less strongly to cell-wall material (*22*, *23*) and/or are more resistant to thermal degradation.

Procyanidin Changes during Storage of Blueberry Juices. Changes in procyanidin composition and content during storage at 25 °C are shown in **Table 2**. All procyanidins declined over 6 months of storage, although the losses were much less marked than those that occurred during processing. From 1 to 6 months of storage, levels of mono-, di-, tri-, and tetramers in nonclarified juices declined by 21, 27, 15, and 48%, respectively, while pentamers were not detected after 6 months. The levels of total procyanidins declined 28% during storage. After 6 months of storage, the nonclarified juices retained only 11% of the total procyanidin levels found in frozen berries, with mono- and dimers retained to a greater extent (28% and 33%) than trimers (15%) and tetramers (8%). Consistent with these findings, only 3−10% of the total procyanidins found in frozen berries used for processing were retained in nonclarified blueberry juice after 60 days of storage at 23 °C (*10*).

Similar losses were observed in clarified juices from 1 to 6 months of storage. Levels of mono-, di-, tri-, tetra-, and pentamers declined 44, 61, 55, 42, and 37%, respectively. After 6 months of storage, the clarified juices retained only 8% of the total procyanidins found in frozen berries, with mono- and dimers (19%) being retained to a greater extent than tri- and tetramers (8%) and pentamers (4%). The losses of procyanidins

Table 3. Content of Procyanidin Oligomers (mg/kg FW) in Blueberry Purée as Affected by Processing and 6 Months of Storage at 25 °C[a]

	storage time			
procyanidin	1 day	1 month	3 months	6 months
monomers	22.6 ± 1.3 a (67%)[b]	13.7 ± 1.9 b (51%)	12.0 ± 0.8 b (36%)	5.8 ± 1.5 c (17%)
dimers	49.3 ± 3.6 a (81%)	30.7 ± 4.3 b (41%)	25.4 ± 2.6 b (42%)	12.5 ± 3.7 c (21%)
trimers	21.3 ± 2.4 a (40%)	11.1 ± 1.2 b (21%)	8.4 ± 0.7 bc (16%)	3.4 ± 0.7 c (6%)
tetramers	20.2 ± 2.9 a (34%)	10.3 ± 0.8 b (17%)	7.9 ± 0.5 bc (13%)	3.3 ± 0.7 c (5%)
pentamers	24.5 ± 6.3 a (32%)	10.7 ± 0.8 b (14%)	3.7 ± 2.3 c (5%)	ND
hexamers	14.3 ± 3.7 a (28%)	7.0 ± 0.5 b (14%)	1.6 ± 1.3 c (3%)	ND
heptamers	2.0 ± 0.8 a (12%)	1.3 ± 1.0 a (8%)	ND	ND
total (monomer−heptamer)	154.3 ± 14.6 a (41%)	84.7 ± 8.5 b (23%)	59.0 ± 6.1 bc (16%)	25.0 ± 6.6 c (7%)

[a] Values represent means ± standard error ($n = 5$). Means within rows with different letters are significantly different ($p \leq 0.05$). Oligomers > DP7 were nondetectible in the purées following processing. [b] Values within parentheses represent percent retention compared to frozen berries.

Table 4. Content of Procyanidin Oligomers (mg/kg FW) in Blueberries CS and CW as Affected by Processing and 6 Months of Storage at 25 °C[a]

	storage time			
procyanidin	1 day	1 month	3 month	6 month
		Canned in Syrup		
monomers	31.9 ± 0.9 a (95%)[b]	26.9 ± 1.1 b (80%)	16.6 ± 1. 1 d (49%)	21.5 ± 1.4 c (64%)
dimers	64.7 ± 3.3 a (107%)	63.9 ± 4.6 a (105%)	44.0 ± 5.1 b (72%)	36.9 ± 3.5 b (61%)
trimers	45.3 ± 1.2 a (84%)	29.1 ± 2.4 b (54%)	15.9 ± 2.4 bc (30%)	13.8 ± 4.6 c (26%)
tetramers	39.8 ± 1.3 b (66%)	23.7 ± 1.3 c (24%)	17.9 ± 2.5 c (30%)	9.3 ± 2.4 d (15%)
pentamers	38.2 ± 1.4 b (51%)	17.9 ± 2.0 c (40%)	16.5 ± 1.3 c (22%)	ND
hexamers	18.9 ± 0.9 b (37%)	10.5 ± 0.5 c (20%)	10.2 ± 1.8 c (20%)	ND
heptamers	2.9 ± 0.2 b (18%)	2.9 ± 0.2 b (17%)	2.3 ± 0.2 b (14%)	ND
octamers	4.9 ± 0.3 b (22%)	2.4 ± 0.4 b (11%)	0.7 ± 0.5 b (3%)	ND
total (monomer−octamer)	242.2 ± 8.5 b (65%)	175.6 ± 10.1 c (47%)	128.2 ± 14.7 d (34%)	81.4 ± 5.2 e (22%)
		Canned in Water		
monomers	27.7 ± 1.2 b (82%)	27.6 ± 1.8 b (88%)	17.9 ± 1.4 c (53%)	12.8 ± 1.0 d (38%)
dimers	57.3 ± 3.3 ab (94%)	53.3 ± 4.8 ab (82%)	50.4 ± 4.0 b (43%)	36.3 ± 1.7 c (60%)
trimers	40.4 ± 1.8 b (75%)	35.0 ± 4.3 bc (65%)	22.9 ± 1.6 cd (83%)	20.9 ± 2.7 d (39%)
tetramers	43.3 ± 2.0 b (74%)	35.7 ± 5.4 b (59%)	22.6 ± 1.9 c (38%)	15.7 ± 1.5 c (26%)
pentamers	56.0 ± 3.0 b (72%)	14.9 ± 3.4 c (20%)	16.8 ± 2.1 c (22%)	17.8 ± 4.1 c (24%)
hexamers	39.2 ± 8.8 a (76%)	13.6 ± 0.8 b (27%)	11.4 ± 2.6 b (22%)	8.4 ± 2.7 b (16%)
heptamers	15.8 ± 2.3 ab (95%)	6.7 ± 0.7 bc (40%)	4.6 ± 0.1 c (27%)	3.7 ± 0.7 c (22%)
octamers	17.1 ± 6.5 a (78%)	7.6 ± 4.1 ab (36%)	8.7 ± 3.7 ab (39%)	2.1 ± 1.0 b (9%)
total (monomer−octamer)	292.8 ± 14.6 b (78%)	187.8 ± 16.7 c (50%)	142.8 ± 7.2 cd (38%)	119.8 ± 10.3 c (32%)

[a] Values represent means ± standard error ($n = 5$). Means within rows for each canned product with different letters are significantly different ($p \leq 0.05$). [b] Values within parentheses represent percent retention compared to frozen berries.

in both types of juices during storage may be due to polymerization reactions with anthocyanins. We recently reported that extensive losses of anthocyanins were accompanied by increased polymeric color values in the same set of samples during storage (*11*). Anthocyanins are known to undergo condensation reactions with procyanidins (*24*), which can be mediated by acetaldehyde (*25*), and furfural (*26*), or occur via direct anthocyanin−tannin reactions (*27*, *28*). It is also possible that procyanidins were degraded as a result of endogenous enzyme activities that were not totally inactivated by the blanching treatment. Both peroxidase (POD) and polyphenol oxidase (PPO) are reported to cause degradation of procyanidins (*29*, *30*). Another possibility is that procyanidins bonded to macromolecules, such as proteins and cell-wall polysaccharides, and formed a precipitate during storage. More research is needed to identify the mechanism(s) responsible for procyanidin losses during storage of blueberry juices.

Procyanidin Changes during Processing and Storage of Blueberry Purée. Processing and storage of purées resulted in extensive losses of procyanidins (**Table 3**). After processing, monomers (67%) and dimers (81%) were retained to a greater extent than tri- to hexamers (40−28% retention), while only 12% of the heptamers were retained. The processed purées retained only 41% of total procyanidins compared to levels found in frozen berries. The greater losses of larger oligomers compared to mono- and dimers were consistent with changes observed following blanching, where berries were heated and mashed, and may be explained by preferential binding of the larger oligomers to cell-wall polysaccharides. However, it is also possible that larger oligomers were converted to mono- and dimers in response to thermal treatment. Interestingly, the mono- and dimers showed greater losses during storage than with processing, with only 17 and 21% of the compounds retained after 6 months of storage. Conversely, the larger oligomers (trimers−heptamers) showed greater losses during processing than with storage, but losses were evident over 6 months of storage. Heptamers were not detectible after 3 months, and pentamers−heptamers were not detectible after 6 months. After 6 months, the purées retained only 7% of the total procyanidins present in frozen berries. Similar to our findings, procyanidin monomers (catechin and epicatechin), dimers (B1 and B2), and trimer (C1) were extensively degraded in apple purée over 6 months of storage at 30 °C (*14*).

Procyanidin Changes during Processing and Storage of Canned Blueberries. Changes in procyanidin composition and content of berries canned in water or syrup are shown in **Table 4**. The entire content of the canned products (berries + water and berries + syrup) were blended and analyzed to accurately determine procyanidin losses during processing and storage. The mono- and dimers were well-retained (82−107%) in both berries CW and CS. However, in berries CS, a trend of decreasing retention with increased oligomer size was observed, ranging

from 84% retention of trimers to only 18−22% retention of hepta- and octamers. After processing berries, CS retained only 65% of the total procyanidins found in frozen berries. Our results contrast those of Hong et al. (*13*), who reported that procyanidins were stable following processing of canned peaches, with minor losses in the fruit accounted for by migration into the canning syrup. In contrast to berries CS, procyanidins in berries CW were better retained following processing with >70% retention for trimers−octamers. The berries CW retained 78% of the total procyanidins present in frozen berries (**Table 4**).

Similar to results obtained with juices and purées, extensive losses of procyanidins occurred during 6 months of storage at 25 °C. In berries CS, 64, 61, 26, and 15% of the mono-, di-, tri-, and tetramers were retained after 6 months of storage, respectively, whereas pentamers−octamers were not detectible. After 6 months of storage, only 22% of the total procyanidins found in frozen berries were retained. A time-related loss in higher oligomers during storage of canned peaches was also observed by Hong et al. (*13*), with oligomers larger than trimers not detected after 3 months of storage.

In berries CW, 38, 60, and 39% of mono-, di-, and trimers were retained after 6 months, while the larger oligomers were retained to a lesser extent. In contrast to berries CS, pentamers−octamers were still detectible in berries CW after 6 months, albeit at very low concentrations. After 6 months of storage, berries CW retained only 32% of the total procyanidins found in frozen berries.

Processing of blueberries caused an overall loss in total procyanidins, but monomers−trimers were generally better retained than higher oligomers. Retention of procyanidins in processed blueberry samples stored for 6 months was better with canning (22−30%) as opposed to juices (8−11%) or purée (7%). However, with canning, we do not know how much might have leached into the water or syrup. Studies are needed to determine if the losses in procyanidins during processing have major implications in health outcomes associated with procyanidin consumption.

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Received for review September 26, 2008. Revised manuscript received December 11, 2008. Accepted January 13, 2009. The project was supported by the National Research Initiative of the USDA Cooperative State Research, Education, and Extension Service (Grant 2005-35503-15409). Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may be suitable.

JF803015S