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Processing and Storage Effects on the Ellagitannin Composition of Processed Blackberry Products

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Changes in blackberry ellagitannin composition in response to juicing (clarified and nonclarified), pureeing, canning (in water or syrup), and freezing as well as changes in processed products during 6 months of storage were investigated. Canning, pureeing, and freezing had little effect on ellagitannins, but processing berries into nonclarified and clarified juices resulted in total ellagitannin losses of 70 and 82%, respectively, due to removal of ellagitannin-rich seeds in the presscake. Minimal changes in total ellagitannin content were observed during storage of thermally processed products, but compositional changes indicative of ellagitannin depolymerization were apparent. The ellagitannin content and composition of frozen berries remained stable over 6 months of storage. Ellagitannins are well retained in canned, pureed, and frozen blackberries, but methods are needed to prevent losses during juice processing and/or exploit the ellagitannin-rich coproducts.

KEYWORDS: Blackberry; ellagitannins; processing; storage

INTRODUCTION

Ellagitannins are an important class of phenolics that have received attention recently due to their purported health benefits (*1*−*3*). Ellagitannins are polymers of hexahydroxydiphenic acid that can be hydrolyzed to form ellagic acid. Blackberries contain a complex mixture of ellagitannins (**Figure 1**) that range in molecular weight from 302.2 (ellagic acid) to 3738.8 (lambertianin D) (*4*,*5*). The ellagitannins in blackberries are located predominantly in the seeds (*4*,*6*), but some are found in the torus (receptacle tissue), and only a few are present in the flesh (*4*). Several studies have evaluated the ellagitannin content in blackberries with values ranging from 8 to >70 mg/100 g of FW (*7*−*9*). There are no current studies on processed blackberries that report the amount of ellagitannins in their native forms. However, one study (*10*) reported that ellagic acid in raspberry jam (not hydrolyzed from ellagitannins) increased 2-fold with processing and 3-fold over 6 months of storage, presumably as a result of ellagitannin depolymerization. This suggests that evaluation of native ellagitannin content changes may offer a greater understanding of the potential bioavailability of these phenolics, because they range in molecular weight and number of hexahydroxydiphenic acid moieties.

Marked losses of anthocyanins can occur when blackberries are processed into juices, purees, and canned products, as a result of thermal degradation, oxidative reactions, and binding to insoluble components (*11*−*13*), but information is lacking on how processing and storage of processed products affect the ellagitannin composition and content of blackberries. Studies have indicated that ellagic acid composition (from hydrolyzed ellagitannins) of other berry fruits can be altered by processing and storage (*10*, *14*, *15*). Freezing strawberries and raspberries resulted in a 20% loss in total ellagic acid (after acid hydrolysis of ellagitannins) with 9 months of storage further exacerbating losses (with up to 40% additional degradation of ellagic acid); however, with 9 months of storage of strawberry jams there were no losses in total ellagic acid observed (*15*). Because there are considerable changes that can occur to the native ellagitannins (i.e., degradation or depolymerization), the effects of processing on ellagitannin composition and content should also prove valuable to understanding their role in health promotion. Thus, the objective of this study was to evaluate the effects of processing and storage on the ellagitannin content of IQF berries and juiced, canned-in-syrup (CS), canned-in-water (CW), and pureed blackberry products.

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MATERIALS AND METHODS

Reagents and Standards. Ellagic acid was purchased from Sigma Aldrich (St. Louis, MO). All solvents (HPLC grade) were obtained from EMD Biosciences (Madison, WI).

Materials. Blackberries (cv. Apache) harvested fully ripe were obtained from the University of Arkansas Agriculture Experiment Station (Clarksville, AR). Berries were stored at −20 °C for <1 month prior to processing.

Processing and Storage. Blackberries were processed into nonclarified and clarified juices, pureed, canned (water or syrup), and individually quick frozen (IQF) products, as previously described (*12*). A flow diagram of juice processing with sampling points identified (1−8) is shown in **Figure 2**. Juices, purees, and canned products were stored at 25 ± 2 °C for 6 months, and IQF berries were stored at −20 °C for 6 months.

Sample Extraction. Prior to extraction, fresh and IQF fruits, berries isolated from canned products (water and syrup), and entire contents of canned samples (berries + water and berries + syrup) were blended for

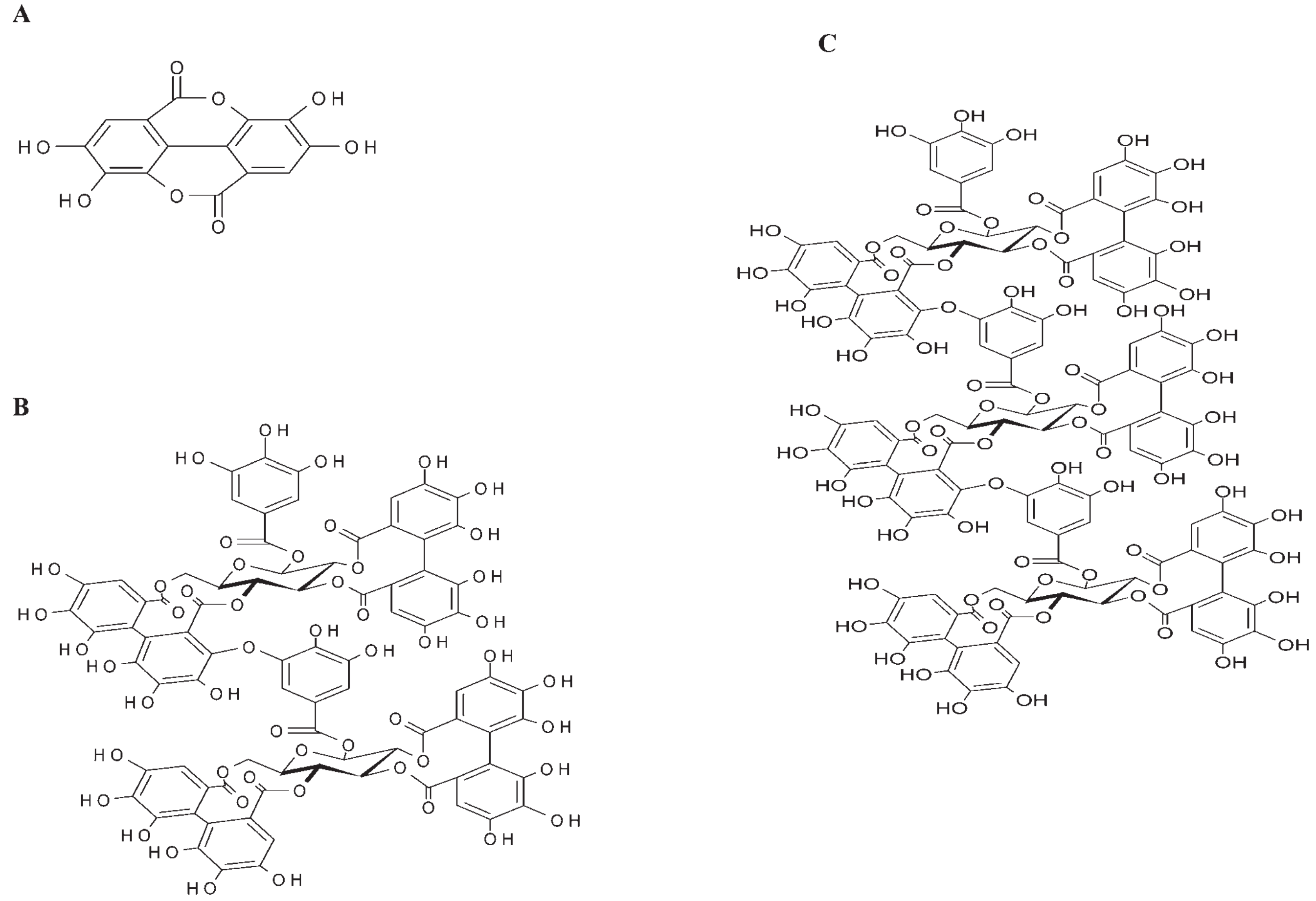

Figure 1. Structures of ellagic acid (**A**) and major ellagitannins, sanguiin H-6/lambertianin A (**B**) and lambertianin C (**C**), found in blackberries.

1 min on high speed in a Black and Decker food processor (Towson, MD). Puree and juice samples required no pre-extraction step.

Blended samples (10 g) of each product were homogenized with 20 mL of acetone/water/acetic (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 (Calboichem, La Jolla, CA), the filter cakes were isolated, and the extraction was repeated. The filtrates were adjusted to a final volume of 100 mL with acetone/water/acetic acid extraction solvent and stored at −70 °C until analysis.

HPLC Analysis of Ellagitannins. Sample extracts (3 mL) were dried using a Speed Vac concentrator (ThermoSavant, Holbrook, NY) and resuspended in 0.5 mL of extraction solvent. The reconstituted samples were passed through 0.45 μm PTFE syringe filters (Varian, Inc., Palo Alto, CA) prior to HPLC analysis. The ellagitannins were analyzed on a Waters Alliance HPLC system (Milford, MA) equipped with a Waters model 996 photodiode array detector and Millenium version 3.2 software (Waters Corp., Milford, MA). Separation was performed using a Phenomenex Aqua 5 μm C_{18} (250 × 4.6 mm) column (Torrance, CA) with a binary gradient of 2% acetic acid for mobile phase A and 0.5% acetic acid in water/acetonitrile (1:1 v/v) for mobile phase B at a flow rate of 1.0 mL/min. A linear gradient was run from 10 to 55% B (0−50 min), from 55 to 100% B (50−60 min), and from 100 to 10% B (60−65 min). The ellagitannins were identified on the basis of comparison of HPLC retention times to our previous HPLC results obtained using the same cultivar, identical HPLC conditions, and LC-MS analysis (*4*). The ellagitannin peaks were quantified as ellagic acid (EA) equivalents using external calibration curves of ellagic acid, with results expressed as milligram EA equivalents per 100 g of fresh berry weight.

Calculations. For blended-canned samples, juices, and purees, the ellagitannins were converted to fresh berry weight using the calculations previously described (*12*).

Statistical Analysis. All data were presented as means ± standard error of five samples taken from each product at each sampling time (fresh, 1 day, and 1, 3, and 6 months), with the exception of frozen berries, which were not sampled at 1 month. The Shapiro−Wilk test was utilized to ensure that sample replicates were normally distributed. Most samples were normally distributed (with $\alpha = 0.05$). The effects of processing and storage on ellagitannin content were analyzed by one-way analysis of variance (JMP software v. 6.0, Cary, NC) to determine significant differences ($p \leq 0.05$) between means.

RESULTS AND DISCUSSION

Freezing Effects on Ellagitannin Content. The ellagitannins quantified by HPLC included two isomers of pedunculagin (Ped, MW = 784.2), two isomers of castalagin/vescalagin (cast/vesc, MW = 933.1), two isomers of lambertianin C (lambC, MW = 2804.2), two isomers of sanguiin H-6/lambertianin A (sangH-6/lambA, MW = 1870.2), one isomer of lambertianin D (lambD, MW = 3738.8), and one isomer of galloylbisHHDPglucose (GalHHDPGlu, MW = 936.2). The fresh fruit concentrations of lambC and lambD were statistically lower than those of the IQF fruit at 1 day (**Table 1**). However, for all other ellagitannins and total ellagitannin content, the differences were not statistically significant. Thus, although there appeared to be a slight increase in extractability of the high molecular weight ellagitannins, overall there was no difference in ellagitannin content due to freezing. de Ancos et al. (*16*) also reported no changes in total acid-hydrolyzed ellagitannin content (reported as ellagic acid) after freezing of raspberry fruit, and Mullen et al. (*17*) reported relatively minor changes in lambC, sangH-6/lambA, and ellagic acid in red raspberries due to freezing.

Thermal Processing Effects on Ellagitannin Content. Thermal processing did not appear to degrade ellagitannins appreciably; however, juicing resulted in significant ellagitannin losses, likely due to the exclusion of the ellagitannin-rich seeds (**Tables 1** and **2**).

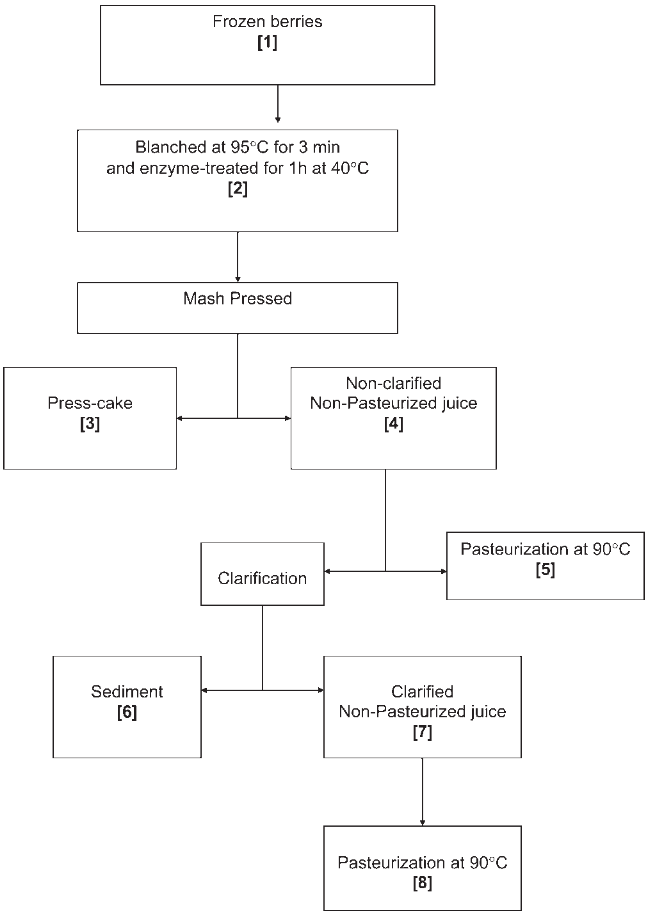

Figure 2. Flow diagram of juice processing with the sampling points identified (1−8).

The total ellagitannin content of blended canned products (CS and CW) and puree was unaffected by thermal processing. Interestingly, there was a slight increase in cast/vesc and ped isomers in the canned and pureed products, suggesting either depolymerization of the larger molecular weight compounds with processing or enhanced extraction due to thermal treatment of the seeds. Although minimal work has been done on processing effects on ellagitannin content, one study (*18*) evaluated the acid-hydrolyzed ellagitannin content (reported as ellagic acid) in blackberry jams as compared to fresh fruit and found that the total ellagic acid content was unchanged by processing, confirming the results from canned and pureed products in this study.

Losses in total ellagitannins in juices were dramatic, with 82 and 70% lost in clarified and nonclarified juices, respectively, compared to IQF fruit used for processing (**Table 2**). With 67% of the original ellagitannin content retained in the presscake, the exclusion of the seeds, where most of the ellagitannins are located (*4*, *6*), accounted for the dramatic losses in ellagitannins in the final product. The concentration of all ellagitannins in the blanched and enzyme-treated mash was 43% greater than that in the frozen fruit, suggesting that the blanching step in combination with the enzyme treatment enhanced the extractability of the ellagitannins, although the other thermal processing treatments discussed previously did not result in increased extractability. According to Rommel and Wrolstad (*14*), the ellagic acid content of raspberry juices was highly affected by the processing technique, particularly the extent of heating, which dramatically increased total ellagic acid due to enhanced extractability from the cell wall matrix. Pasteurization of nonclarified and clarified juices did not affect levels of total ellagitannins, indicating that the compounds are heat stable.

Storage Effects on Ellagitannin Content. In nonclarified juices and blended canned-in-syrup products, losses in total ellagitannins occurred throughout storage (**Tables 3** and **4**). In contrast,

Table 1. Ellagitannin Content (Milligrams per 100 g of FW Original Berry) of Fresh and Individually Quick Frozen (IQF) Fruit and Canned-in-Syrup (CS), Canned-in-Water (CW), Pureed, and Clarified and Nonclarified Juice Products As Affected by Processing (1 Day)[a]

product	ped	cast/vesc	lambC	sangH-6/lambA	lambD	galHHDP-glu	total
fresh	0.11 ± 0.02 cd	0.04 ± 0.01c	9.12 ± 1.03c	9.78 ± 0.54b	2.24 ± 0.20bc	2.06 ± 0.27ab	23.34 ± 2.01bc
IQF	0.14 ± 0.02bc	0.04 ± 0.01c	15.20 ± 3.69ab	12.77 ± 2.76ab	4.52 ± 1.36a	2.50 ± 0.60a	35.16 ± 8.18ab
CS blended	0.20 ± 0.02a	0.10 ± 0.00a	16.25 ± 0.46ab	16.66 ± 0.87a	3.28 ± 0.18ab	2.97 ± 0.16a	39.48 ± 1.42a
CW blended	0.18 ± 0.04ab	0.09 ± 0.00a	20.46 ± 054a	17.67 ± 0.43a	3.68 ± 0.27ab	2.31 ± 0.41a	44.39 ± 1.18a
pureed	0.19 ± 0.03a	0.06 ± 0.01b	12.71 ± 2.34bc	13.78 ± 2.79ab	3.26 ± 0.82ab	2.23 ± 0.43a	32.24 ± 6.39ab
clarified juice	0.05 ± 0.00d	0.03 ± 0.00c	2.42 ± 0.22d	2.44 ± 0.18c	0.39 ± 0.05c	1.06 ± 0.12b	6.39 ± 0.51d
nonclarified juice	0.06 ± 0.00d	0.03 ± 0.00c	2.88 ± 0.08d	4.28 ± 0.22c	1.96 ± 0.11bc	6.39 ± 0.51d	10.41 ± 0.39 cd

[a] Values represent means ± SEM ($n = 5$). Means within columns with different letters are significantly different ($p \leq 0.05$). Abbreviations: ped, pedunculagin isomers; cast/vesc, castalagin/vescalagin isomers; lambC, lambertianin C isomers; sangH-6/lambA, sanguiin H-6/lambertianin A isomers; lambD, lambertianin D isomers; galHHDPGlu, galloylbisHHDPglucose isomers.

Table 2. Ellagitannin Content (Milligrams per 100 g of FW Original Berry) Throughout Juice Processing with Each Processing Step Corresponding to Steps Indicated in **Figure 1**[a]

processing step	ped	cast/vesc	lambC	sangH-6/lambA	lambD	galHHDPglu	total
[1] frozen	0.14 ± 0.02c	0.04 ± 0.01c	15.20 ± 3.69b	12.77 ± 2.76b	4.52 ± 1.36a	2.50 ± 0.60a	35.16 ± 8.18b
[2] blanched	0.31 ± 0.03a	0.13 ± 0.01a	22.67 ± 0.56a	18.13 ± 0.55a	6.14 ± 0.63a	2.88 ± 0.28a	50.24 ± 1.62a
[3] presscake	0.22 ± 0.03b	0.08 ± 0.01b	11.47 ± 0.35b	8.35 ± 0.25c	2.45 ± 0.10b	0.98 ± 0.03b	23.55 ± 0.66c
[4] juice, NC, NP	0.07 ± 0.00d	0.03 ± 0.00c	3.43 ± 0.11c	4.54 ± 0.19d	1.71 ± 0.09bcd	1.36 ± 0.05b	10.91 ± 0.40d
[5] juice, NC, P	0.06 ± 0.00d	0.03 ± 0.00c	2.88 ± 0.08c	4.28 ± 0.22de	1.96 ± 0.10bc	4.43 ± 0.30b	10.41 ± 0.39de
[6] sediment	0.00 ± 0.00e	0.00 ± 0.00d	0.10 ± 0.00c	0.06 ± 0.00f	0.02 ± 0.00d	0.01 ± 0.00c	0.20 ± 0.00e
[7] juice, C, NP	0.07 ± 0.01d	0.03 ± 0.00c	1.39 ± 0.04c	1.20 ± 0.08ef	0.84 ± 0.07bcd	0.60 ± 0.10bc	4.13 ± 0.23de
[8] juice, C, P	0.05 ± 0.00d	0.03 ± 0.00c	2.42 ± 0.22c	2.44 ± 0.18def	0.40 ± 0.05 cd	1.06 ± 0.12b	6.39 ± 0.51de

[a] Values represent means ± SEM ($n = 5$). Means within columns with different letters are significantly different ($p \leq 0.05$). Abbreviations: ped, pedunculagin isomers; cast/vesc, castalagin/vescalagin isomers; lambC, lambertianin C isomers; sangH-6/lambA, sanguiin H-6/lambertianin A isomers; lambD, lambertianin D isomers; galHHDPglu, galloylbisHHDPglucose isomers; NC, nonclarified; NP, nonpasteurized; P, pasteurized; C, clarified.

Table 3. Ellagitannin Content (Milligrams per 100 g of FW Original Berry) of Clarified and Nonclarified Juices As Affected by Storage Time[a]

	ped	cast/vesc	lambC	sangH-6/lambA	lambD	galHHDPglu	total
				Nonclarified			
1 day	0.06 ± 0.00a	0.03 ± 0.00a	2.88 ± 0.08a	4.28 ± 0.22a	1.96 ± 0.11a	1.19 ± 0.06ab	10.41 ± 0.39a
1 month	0.06 ± 0.01a	0.04 ± 0.00a	3.17 ± 0.08a	3.05 ± 0.11b	0.89 ± 0.06b	1.04 ± 0.04b	8.26 ± 0.24b
3 months	0.07 ± 0.01a	0.04 ± 0.00a	1.93 ± 0.23b	2.13 ± 0.27c	0.48 ± 0.05c	1.47 ± 0.19a	6.12 ± 0.65c
6 months	0.05 ± 0.01a	0.03 ± 0.00a	1.67 ± 0.07b	2.33 ± 0.10c	0.55 ± 0.04c	1.36 ± 0.05ab	5.99 ± 0.20c
				Clarified			
1 day	0.05 ± 0.00b	0.03 ± 0.00b	2.42 ± 0.22a	2.44 ± 0.18a	0.39 ± 0.05ab	1.06 ± 0.12a	6.39 ± 0.51a
1 month	0.06 ± 0.01ab	0.03 ± 0.00b	2.02 ± 0.26a	2.43 ± 0.44a	0.65 ± 0.25a	1.08 ± 0.15a	6.28 ± 1.07a
3 months	0.07 ± 0.01a	0.04 ± 0.00a	1.50 ± 0.10b	2.03 ± 0.18a	0.66 ± 0.06a	1.28 ± 0.13a	5.59 ± 0.45a
6 months	0.06 ± 0.01ab	0.03 ± 0.00b	1.26 ± 0.08b	1.84 ± 0.11a	0.29 ± 0.03b	1.24 ± 0.07a	4.73 ± 0.28a

[a] Values represent means ± SEM (n = 5). Means within columns and treatment groups with different letters are significantly different ($p \leq 0.05$). Abbreviations: ped, pedunculagin isomers; cast/vesc, castalagin/vescalagin isomers; lambC, lambertianin C isomers; sangH-6/lambA, sanguiin H-6/lambertianin A isomers; LambD, lambertianin D isomers; galHHDPglu, galloylbisHHDPglucose isomers.

Table 4. Ellagitannin Content (Milligrams per 100 g of FW Original Berry) of Blended Canned-in-Syrup (CS) and Canned-in-Water (CW) Products As Affected by Storage Time[a]

	ped	cast/vesc	lambC	sangH-6/lambA	lambD	galHHDPglu	total
				CS Blended			
1 day	0.22 ± 0.00a	0.10 ± 0.00a	16.25 ± 0.46a	16.66 ± 0.87a	3.28 ± 0.18ab	2.97 ± 0.16a	39.48 ± 1.42a
1 month	0.19 ± 0.02ab	0.08 ± 0.01b	12.01 ± 1.58ab	13.16 ± 1.81ab	3.73 ± 0.54a	2.56 ± 0.23ab	31.72 ± 4.08ab
3 months	0.15 ± 0.01b	0.07 ± 0.01b	8.42 ± 1.78b	7.15 ± 1.50c	1.51 ± 0.37c	1.33 ± 0.50c	18.62 ± 4.02c
6 months	0.21 ± 0.01a	0.07 ± 0.00b	11.86 ± 2.40ab	11.76 ± 2.34bc	2.53 ± 0.32bc	1.90 ± 0.12bc	28.34 ± 5.05bc
				CW Blended			
1 day	0.18 ± 0.04a	0.09 ± 0.00a	20.46 ± 0.54a	17.67 ± 0.43a	3.68 ± 0.27ab	2.31 ± 0.41ab	44.39 ± 1.18a
1 month	0.16 ± 0.03a	0.07 ± 0.01ab	17.28 ± 1.74ab	16.20 ± 1.48a	3.98 ± 0.46a	2.70 ± 0.37a	40.38 ± 3.78a
3 months	0.14 ± 0.02a	0.06 ± 0.00b	12.10 ± 0.68c	10.85 ± 1.03b	2.36 ± 0.23b	1.03 ± 0.59b	26.54 ± 2.50b
6 months	0.19 ± 0.03a	0.07 ± 0.01ab	15.01 ± 0.50bc	15.32 ± 0.56a	3.15 ± 0.40ab	3.69 ± 0.43a	37.43 ± 1.69a

[a] Values represent means ± SEM (n = 5). Means within columns and treatment groups with different letters are significantly different ($p \leq 0.05$). Abbreviations: ped, pedunculagin isomers; cast/vesc, castalagin/vescalagin isomers; lambC, lambertianin C isomers; sangH-6/lambA, sanguiin H-6/lambertianin A isomers; lambD, lambertianin D isomers; galHHDPglu, galloylbisHHDPglucose isomers.

Table 5. Ellagitannin Content (Milligrams per 100 g of FW Original Berry) of Puree As Affected by Storage Time[a]

	ped	cast/vesc	lambC	sangH-6/lambA	lambD	galHHDPglu	total
1 day	0.19 ± 0.03a	0.06 ± 0.01a	12.71 ± 2.34a	13.78 ± 2.79a	3.26 ± 0.82a	2.23 ± 0.43a	32.24 ± 6.39a
1 month	0.18 ± 0.02a	0.06 ± 0.01a	11.47 ± 1.58a	13.37 ± 1.41a	4.28 ± 0.49ab	2.39 ± 0.20a	31.74 ± 3.49a
3 months	0.19 ± 0.02a	0.06 ± 0.00a	13.10 ± 0.69a	12.91 ± 0.60a	2.97 ± 0.26ab	2.60 ± 0.13a	31.82 ± 1.47a
6 months	0.19 ± 0.03a	0.06 ± 0.01a	14.01 ± 0.58a	14.38 ± 0.86a	2.55 ± 0.18b	2.58 ± 0.15a	33.77 ± 1.66a

[a] Values represent means ± SEM (n = 5). Means within columns with different letters are significantly different ($p \leq 0.05$). Abbreviations: ped, pedunculagin isomers; cast/vesc, castalagin/vescalagin isomers; lambC, lambertianin C isomers; sangH-6/lambA, sanguiin H-6/lambertianin A isomers; LambD, lambertianin D isomers; galHHDPglu, galloylbisHHDPglucose isomers.

there were no changes in total ellagitannins in clarified juices, blended CW, or pureed products during 6 months of storage (**Tables 4** and **5**). In blended CS products, there were 29% losses in sangH-6/lambA, but ped, cast/vesc, lambC, and lambD levels remained stable. In blended CW products, there were 27% losses in lambC, but levels of all other ellagitannins remained stable. In nonclarified juices, there were 42% losses in lambC, 46% losses in sangH-6/lambA, and 72% losses in lambD during storage; however, there were minimal changes in ped and cast/vesc with storage. In clarified juices, there were only minor losses in lambC, whereas all other ellagitannins were unchanged by storage. The greater losses of ellagitannins in nonclarified versus clarified juices may be due to binding of ellagitannins to insoluble materials, that is, cell wall polysaccharides and/or proteins. Tannins have been shown to bind irreversibly to macromolecules through hydrogen bonding and hydrophobic interactions (*19*, *20*), and ellagitannins have been recovered from the sediments formed during storage of blackberry juice (*21*), green tea (*22*), and Muscadine juice and wine (*23*). Overall, the major losses in nonclarified juices and canned products were due to losses in higher molecular weight ellagitannins (i.e., sangH-6/lambA, MW = 1870.2; lambC, MW = 2804.2; and lambD, MW = 3738.8). A similar trend was observed in puree products, although total ellagitannin content remained unchanged, there was a 22% loss in lambD over 6 months of storage.

While analyzing the same extracts for flavonol content on the Aqua C_{18} column, we discovered by LC-MS analysis that ellagic acid coeluted with quercetin 3-galactoside (data not shown). The peak area for the coeluting compounds increased significantly (1.2−3.2-fold) in juices, puree, and canned berries over 6 months of storage, but was stable in IQF fruit during storage. Because all other flavonols changed little during storage, we believe this change reflects depolymerization of high molecular weight ellagitannins, sangH-6/lambA, MW = 1870.2, lambC, MW = 2804.2,

Table 6. Ellagitannin Content (Milligrams per 100 g of FW Original Berry) of IQF Berries As Affected by Storage Time[a]

	ped	cast/vesc	lambC	sangH-6/lambA	lambD	galHHDPglu	total
1 day	0.14 ± 0.02a	0.04 ± 0.01a	15.20 ± 3.69a	12.77 ± 2.76a	4.52 ± 1.36a	2.50 ± 0.60b	35.16 ± 8.18a
3 months	0.13 ± 0.02a	0.05 ± 0.01a	12.97 ± 0.93a	13.72 ± 0.90a	2.95 ± 0.43a	3.25 ± 0.19ab	33.06 ± 2.28a
6 months	0.17 ± 0.03a	0.06 ± 0.01a	17.19 ± 2.20a	15.82 ± 0.90a	4.08 ± 0.27a	4.43 ± 0.30a	41.75 ± 2.96a

[a] Values represent means ±standard error ($n = 5$). Means within columns with different letters are significantly different ($p \leq 0.05$). Abbreviations: ped, pedunculagin isomers; cast/vesc, castalagin/vescalagin isomers; lambC, lambertianin C isomers; sangH-6/lambA, sanguiin H-6/lambertianin A isomers; LambD, lambertianin D isomers; galHHDPglu, galloylbisHHDPglucose isomers.

Table 7. Ellagitannin Content (Milligrams in the Whole Can) of Canned-in-Syrup (CS) and Canned-in-Water (CW) Berry and Liquid Fractions As Affected by Storage Time[a]

	ped	cast/vesc	lambC	sangH-6/lambA	lambD	galHHDPglu	total
				CS Berries			
1 day	0.45 ± 0.01a	0.15 ± 0.00a	51.01 ± 1.73a	40.77 ± 1.16a	15.11 ± 0.61a	7.21 ± 0.36a	130.61 ± 2.21a
1 month	0.33 ± 0.07ab	0.12 ± 0.02b	35.02 ± 2.91b	30.77 ± 1.85b	4.85 ± 0.34b	5.86 ± 0.35ab	88.29 ± 6.53b
3 months	0.34 ± 0.01ab	0.11 ± 0.00b	10.08 ± 6.19c	8.26 ± 5.05c	1.69 ± 1.02c	4.62 ± 0.47b	76.50 ± 5.49b
6 months	0.31 ± 0.04b	0.10 ± 0.01b	27.71 ± 2.66b	27.35 ± 2.68b	5.64 ± 0.57b	4.41 ± 0.62b	76.16 ± 6.89b
				CS Liquid			
1 day	0.05 ± 0.01b	0.01 ± 0.00c	3.54 ± 0.44b	0.83 ± 0.20c	1.09 ± 0.18b	1.23 ± 0.10c	7.49 ± 0.74c
1 month	0.08 ± 0.01b	0.03 ± 0.00bc	3.52 ± 0.63b	1.80 ± 0.28ab	1.28 ± 0.37b	0.85 ± 0.14d	9.90 ± 1.37c
3 months	0.15 ± 0.00a	0.05 ± 0.00ab	4.09 ± 0.32b	1.35 ± 0.10bc	1.34 ± 0.20b	1.81 ± 0.06b	13.33 ± 0.57b
6 months	0.18 ± 0.02a	0.05 ± 0.01a	6.13 ± 0.17a	2.02 ± 0.13a	2.12 ± 0.12a	2.23 ± 0.04a	18.29 ± 0.86a
				CW Berries			
1 day	0.31 ± 0.03ab	0.15 ± 0.01a	30.25 ± 9.56b	28.91 ± 9.75a	9.76 ± 3.38a	3.28 ± 1.83a	84.32 ± 24.21a
1 month	0.31 ± 0.05ab	0.13 ± 0.01ab	45.87 ± 2.55a	35.68 ± 1.31a	9.31 ± 0.57a	3.98 ± 1.31a	106.53 ± 2.89a
3 months	0.28 ± 0.04b	0.11 ± 0.01b	30.78 ± 0.91b	28.96 ± 1.30a	6.76 ± 0.34a	5.58 ± 0.70a	82.27 ± 3.09a
6 months	0.40 ± 0.03a	0.14 ± 0.00a	42.60 ± 3.31ab	41.61 ± 1.59a	8.26 ± 1.00a	6.65 ± 0.30a	113.86 ± 6.69a
				CW Liquid			
1 day	0.05 ± 0.02b	0.02 ± 0.01b	2.77 ± 0.25a	0.72 ± 0.15b	0.91 ± 0.07b	1.04 ± 0.15a	6.76 ± 1.28b
1 month	0.10 ± 0.04ab	0.04 ± 0.02ab	3.38 ± 0.72a	1.94 ± 0.43a	0.67 ± 0.08b	0.96 ± 0.46a	10.27 ± 2.48ab
3 months	0.11 ± 0.00ab	0.04 ± 0.00ab	2.67 ± 0.01a	0.90 ± 0.06b	0.86 ± 0.03b	1.16 ± 0.03a	9.44 ± 0.13ab
6 months	0.15 ± 0.00a	0.06 ± 0.00a	3.63 ± 0.37a	1.43 ± 0.08ab	1.42 ± 0.15a	1.60 ± 0.08a	13.46 ± 0.67a

[a] Values represent means ± standard error ($n = 5$). Means within columns and treatment groups with different letters are significantly different ($p \leq 0.05$). Abbreviations: ped, pedunculagin isomers; cast/vesc, castalagin/vescalagin isomers; lambC, lambertianin C isomers; sangH-6/lambA, sanguiin H-6/lambertianin A isomers; LambD, lambertianin D isomers; galHHDPglu, galloylbisHHDPglucose isomers.

and lambD, MW = 3738.8, to ellagic acid. Because it is unlikely that compounds over 1000 DA are absorbed to any appreciable extent (*24−26*), the depolymerization of ellagitannins to ellagic acid may result in additional health benefits to consumers. Although the changes in native ellagitannin content over storage have not been evaluated previously, one study reported minimal changes in ellagic acid derivatives throughout storage of raspberry jam (similar to the behavior of low molecular weight ellagitannins in this study), whereas ellagic acid content increased dramatically with storage, suggesting depolymerization of higher molecular weight ETs (*10*).

Overall, the ellagitannin content of IQF berries remained unchanged over 6 months of storage (**Table 6**). There were no changes in individual ellagitannins with the exception of GalHHDPGlu, which increased by 44%. Thus, the ellagitannin content of frozen fruit was very stable throughout storage with relatively no degradation or depolymerization of ellagitannins. These results contrast to the 30−40% losses of ellagic acid in frozen raspberries and strawberries over 9 months of storage reported previously (*15*). However, consistent with our findings, minimal changes in ellagic acid content (after acid hydrolysis of ellagitannins) of frozen blackberries over 12 months of storage were reported by Gonzalez et al. (*27*).

Ellagitannin Content of Canned Berries and Cover Liquid. By evaluating the content of ellagitannins in the berries and liquid canning medium fractions separately (expressed as mg in the whole can) throughout storage, we were able to quantify the amount of ellagitannins that leached out of the berries into the liquid canning media during storage (**Table 7**). The ellagitannin content of CS berries decreased dramatically throughout 6 months of storage (42% loss in total ellagitannins), whereas the total ellagitannin content of the CS liquid fraction increased 2.4-fold from 1 day to 6 months of storage. In contrast, there was no change in the CW berry total ellagitannin content with storage due to large variations in total content, but the total ellagitannin content of the CW liquid fraction increased 2.0-fold from 1 day to 6 months of storage, indicating leaching still occurred. Individual ellagitannins behaved similarly throughout storage in all samples. The loss of liquid due to draining of canned products by consumers may be a source of losses in ellagitannins, but even with increased ellagitannin content of the liquid fractions over 6 months of storage, there were still only 10−20% of the total ellagitannins present in the liquid fractions. The remaining 80−90% of total ellagitannins was retained in the berries; therefore, the effects of draining the product would be minimal and may not reduce potential health benefits from ellagitannins.

In summary, the ellagitannin contents of canned, pureed, and frozen products were relatively unaffected by processing; however, the total ellagitannin concentration of juices changed markedly during juice processing steps. Ellagitannin content increased appreciably following blanching, indicating that tissue softening facilitated extraction. The low levels of ellagitannins in juice products were attributed to loss of the presscake, which resulted in the exclusion of seeds, the primary source of ellagitannins in blackberry fruit. Although the juices are not a significant source of ellagitannins in the diet, the presscake may prove to be valuable for other nutraceutical applications.

The storage of all thermally processed products resulted in compositional changes in ellagitannins, although minimal changes in total ellagitannin content were observed. Overall, it appeared as though there were lower levels of high molecular weight ellagitannins (> 1000 Da) over time. This, coupled with the apparent large increases in ellagic acid indicates that depolymerization of ellagitannins occurred during long-term storage at room temperature.

ABBREVIATIONS USED

Ped, pedunculagin isomers; cast/vesc, castalagin/vescalagin isomers; lambC, lambertianin C isomers; sangH-6/lambA, sanguiin H-6/lambertianin A isomers; lambD, lambertianin D isomers; GalHHDPGlu, galloylbisHHDPglucose isomers; EA, ellagic acid.

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