JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Review

pubs.acs.org/JAFC

Processing and Storage Effect on Berry Polyphenols: Challenges and Implications for Bioactive Properties

Luke R. Howard,*[,†] Ronald L. Prior,[†] Rohana Liyanage,[§] and Jackson O. Lay[§]

[†]Department of Food Science, University of Arkansas, 2650 North Young Avenue, Fayetteville, Arkansas 72704, United States

[§]University of Arkansas Statewide Mass Spectrometry Facility, 1260 West Maple Street, Fayetteville, Arkansas 72701, United States

ABSTRACT: Anthocyanins and tannins in blueberries, blackberries and black raspberries are susceptible to degradation during processing, with juices showing the greatest losses due to physical removal of skins and seeds. Anthocyanins and procyanidins are also degraded in processed products stored at ambient temperature with losses accompanied by increased polymeric pigments (PPs). Using chokeberry as a model, formation of PPs occurred in both pasteurized and aged juices and pasteurized juice contained a greater proportion of low molecular weight PPs than aged juice, while aged juice contained a greater proportion of higher molecular PPs. Formation of PP accounts for some of the losses of anthocyanins and procyanidins during processing and storage, but the complete fate of anthocyanins remains unclear. In this review we highlight the steps in processing where significant losses of polyphenols occur, and discuss potential mechanisms responsible for losses, methods to mitigate losses, and implications on bioactive properties.

KEYWORDS: *anthocyanins, black raspberry, blackberry, blueberry, chokeberry, ellagitannins, polymeric pigments, processing, procyanidins, storage*

INTRODUCTION

Berries have received much attention recently due to a myriad of health-promoting properties and have been the subject of two proceedings published in the *Journal of Agricultural and Food Chemistry*.[1,2] Blueberries, blackberries, and black raspberries in particular vary greatly in polyphenol composition and concentration, the compounds thought to play a major role in health promotion. The intense blue to black pigmentation in these berries is due to various compositions and concentrations of anthocyanins. Blueberries have the most complex anthocyanin profile among common berries, containing over 25 individual anthocyanins, and are unique compared to other berries in that three sugar moieties, glucose, galactose, and arabinose, are found linked to five anthocyanidins, delphinidin (Dpd), cyanidin (Cyd), petunidin (Ptd), peonidin (Pnd), and malvidin (Mvd), in addition to acylated derivatives.[3−5] Blackberries consist mainly of cyanidin glycosides, with cyanidin glucoside predominating.[6−8] Black raspberries are also rich in cyanidin glycosides, with cyanidin rutinoside predominating, but the berries also contain appreciable levels of cyanidin 3-sambubioside-5-rhamnoside, cyanidin glucoside, and cyanidin sambubioside.[5,9] In addition to their role as natural pigments, berry anthocyanins have been the focus of many studies on health-promoting effects related to the prevention of chronic diseases including heart disease, cancer, and obesity.[10−21]

Blueberries, blackberries, and black raspberries also vary in composition of hydrolyzable (ellagitannins) and condensed (procyanidins) tannins. Blueberries contain a diverse array of procyanidins ranging from monomeric flavanols to oligomers (degree of polymerization (DP) 2−10) to high molecular weight polymers (DP > 10),[22] but are devoid of ellagitannins. In contrast, blackberries and black raspberries contain both ellagitannins and procyanidins, but ellagitannins are present in much higher concentrations. Ellagitannins and ellagic acid conjugates have been characterized in blackberries, with lambertianin C and sanguiin H6 being the major ellagitannins present in the berries.[23,24] Little information is available on ellagitannin composition of black raspberries, but lambertianin C and sanguiin H6 appear to predominate.[25]

Most studies reporting on the health-promoting effects of berries have been carried out using fresh or freeze-dried fresh berries or flavonoid-rich extracts isolated from fresh berries. Unfortunately, due to their perishable nature and limited seasonal availability, berries are commonly consumed in various processed forms including juices, purees, jams, nectars, and canned products. It is well-known that polyphenols, especially anthocyanins, are susceptible to degradation during processing, and, in general, processes involving more steps such as juicing result in the greatest losses.[26] Although most studies involving berry polyphenols have focused on the effects of processing, the polyphenol losses incurred during storage of products at ambient temperature can often be more severe than those incurred during processing. This is especially true in the case of anthocyanins, which are susceptible to polymerization reactions with procyanidins during processing (commonly referred to as polymeric pigments) but to a larger degree during long-term storage. The transformation of monomeric anthocyanins to polymeric pigments (PPs) in processed berry products not only affects the color of the products but also may have major

Special Issue: Food Bioactives and the Journal of Agricultural and Food Chemistry

Received: November 14, 2011
Revised: January 11, 2012
Accepted: January 13, 2012
Published: January 13, 2012

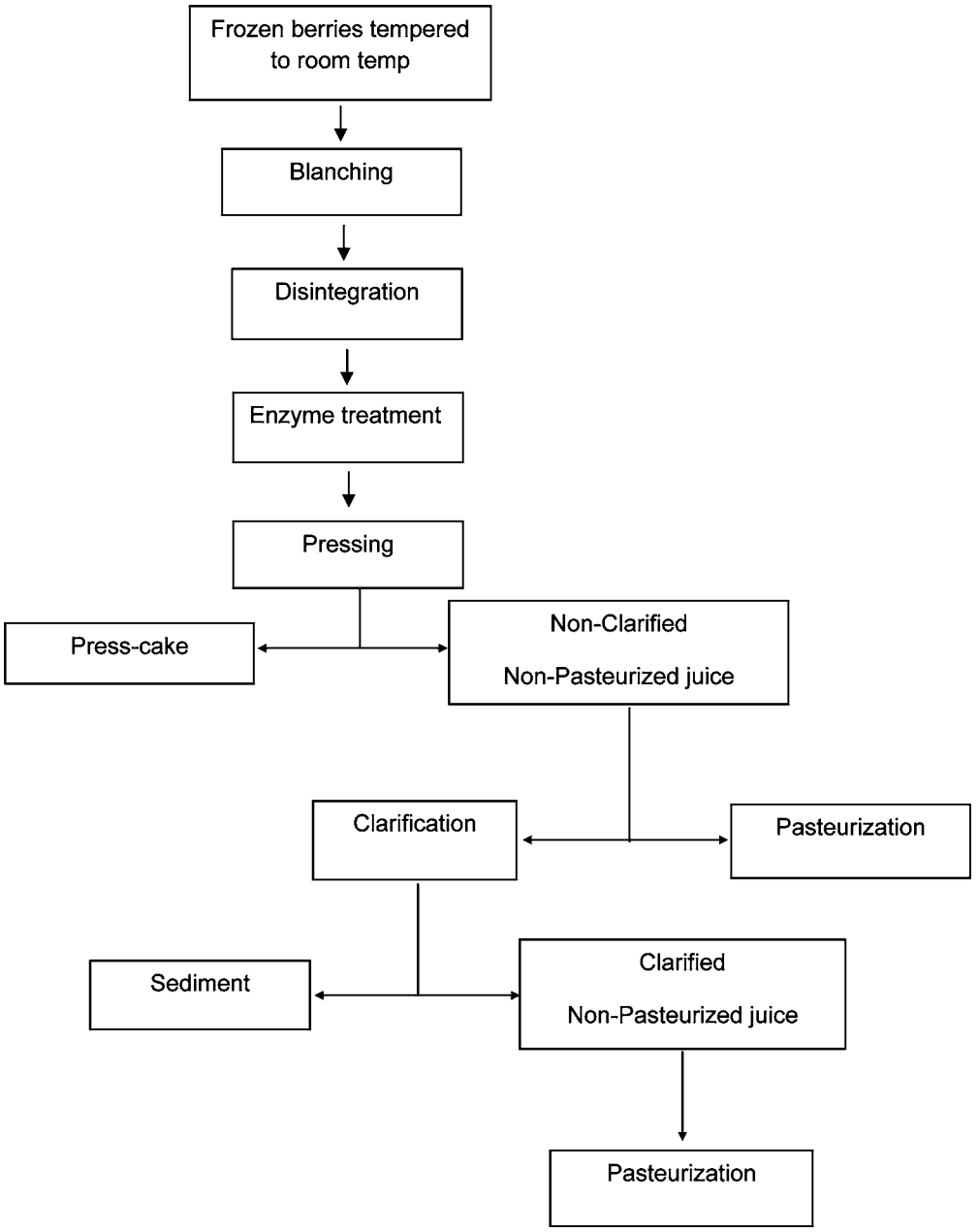

Figure 1. Schematic of juice processing steps.

implications on health benefits associated with anthocyanins and procyanidins. The goal of this review is to (1) highlight the steps in processing where significant losses of anthocyanins and tannins (procyanidins and ellagitannins) occur, (2) describe potential mechanisms responsible for anthocyanin and tannin losses, (3) discuss potential methods/treatments to mitigate anthocyanin and tannin losses during processing and storage, and (4) discuss potential implications of processing and storage-induced changes on bioactive properties associated with anthocyanins and procyanidins.

LOSSES OF ANTHOCYANINS DURING BERRY PROCESSING AND STORAGE

Losses of Anthocyanins during Processing. *Blueberries.* The effect of processing blueberries into juice (clarified and nonclarified), puree, and canned products (water or syrup) was studied by Brownmiller et al.[27] Generally, anthocyanins were better retained in canned berries (66% in water, 72% in syrup) and puree (52%) than in clarified juice (41%), although nonclarified juices retained levels comparable to those of canned berries (72%). The 72% retention of anthocyanins in berries canned in syrup is consistent with retention values of 61, 68, 86, and 116% reported by ref 28 for blueberries canned in syrup. Approximately 10−17% of the anthocyanins diffuse out of the berries into the syrup during canning.[27,28] The effect of steam blanching berries prior to canning was found to reduce anthocyanin levels in the canned berries and increase levels in the syrup as a result of berry softening.[28]

The greater losses of anthocyanins during juice processing compared to other products are expected due to the larger number and complexity of processing steps involved. In juice processing frozen blueberries are typically thawed under refrigeration, blanched (in some studies), milled, depectinized, pressed, and pasteurized (Figure 1). Juices may also be clarified

prior to pasteurization and/or concentrated following pasteurization. Due to the presence of oxidoreductases such as polyphenol oxidase and/or peroxidase, which are thought to play a major role in blueberry anthocyanin degradation,[29−32] an initial blanching treatment to inactivate the enzymes is important. Several studies have compared the effect of steam blanching thawed blueberries versus berries receiving no blanch treatment on anthocyanin retention of juices and found that blanching treatment improves the retention of anthocyanins.[28,33,34] In a study by Brownmiller et al.[27] frozen blueberries were blanched immediately (not allowed to thaw) in a steam kettle to a temperature of 95 °C. By rapidly inactivating the enzymes, 41 and 72% of the anthocyanins were recovered in the clarified and nonclarified juices. We suspect that allowing frozen berries to thaw under refrigeration results in activation of oxidoreductases, which leads to significant degradation of anthocyanins prior to their inactivation by blanching. Depectinization of berry mash with cell wall hydrolytic enzymes used to increase juice yield can also lead to loss of anthocyanins if enzyme preparations are contaminated with glycosidases, which can hydrolyze anthocyanin glycosides to highly unstable aglycones. Buchert et al.[35] compared the effects of different enzyme preparations on anthocyanin composition of bilberry juice and found that the enzyme preparations varied markedly in β-galactosidase, α-arabinosidase, and β-glucosidase activities. The presence of β-galactosidase in all of the enzyme preparations studied resulted in complete loss of Dpd, Cyd, Ptd, Pnd, and Mvd galactosides in the juice. This study illustrates the importance of screening enzyme preparations used in juice processing for glycosidase activities and optimizing the dose to minimize anthocyanin degradation. The pressing step results in extensive losses of anthocyanins due to physical removal of the anthocyanin-rich skins as well as binding of released anthocyanins to cell wall polysaccharides. The amounts of anthocyanins retained in presscake reported in several studies are 15%,[27] 18%,[32] 42% (blanched berries),[33] and 55% (nonblanched berries).[33] It appears blanching, which inactivates oxidoreductases, also facilitates softening of the skins, allowing for greater extraction of anthocyanins. Some juices receive a clarification step to remove insoluble materials, and this process has been shown to result in anthocyanin losses of 8%[33] and 25%,[27] presumably due to binding of anthocyanins to cell wall polysaccharides/proteins. Blueberry juices are commonly pasteurized to destroy spoilage microorganisms and achieve a 5 log pathogen reduction. It is well-known that anthocyanin degradation follows first-order reactions kinetics and that processing at lower temperatures for short times improves their retention.[36] The short pasteurization time used for blueberry juices, typically 60−90 s at 90 °C, generally results in minor (<10%) losses of anthocyanins.[27,32,33,37] Berry juices are also commonly concentrated to ≥65 °Brix through the use of evaporators operated at 40−50 °C under vacuum. Concentrating blueberry juice to 65−73.5 °Brix has been shown to have only a minor effect (<10% loss) on anthocyanins.[32,33] It is clear that anthocyanin losses during blueberry juice processing are mainly due to enzymatic degradation that may occur prior to inactivation by blanching and physical removal of the skins in the presscake, whereas clarification, pasteurization, and concentration steps result in minor losses.

Anthocyanin Composition Changes. *Blueberries.* The various steps involved in juice processing have also been shown to alter anthocyanin composition compared to that of fresh blueberries. Dpd glycosides appear to be most unstable during processing due to extensive hydroxylation on the B-ring followed by Ptd glycosides, whereas Mvd glycosides tend to be most stable due to extensive methoxylation on the B-ring, and Pnd and Cyd glycosides fluctuate among studies.[32,33,38] Skrede et al.[32] reported that the rate of anthocyanin degradation during processing was more related to anthocyanidin structure than to sugar substitution. However, Ichiyangi et al.[39] studied acid-mediated hydrolysis of blueberry anthocyanins and found that the hydrolysis rate of individual anthocyanins was influenced more by type of conjugated sugar than by aglycone structure with the rate constants of anthocyanin hydrolysis in the order arabinoside > galactoside > glucoside.

Blackberries. Anthocyanins were better retained in berries canned in water (82%), berries canned in syrup (89%), and purees (73%) than in nonclarified (34%) and clarified (33%) juices.[40] In a study by Wu et al.,[41] total anthocyanin retentions in Marian and Evergreen blackberries canned in water were 53 and 65%, whereas Marian and Evergreen berries canned in syrup retained only 30%. They also reported that Marion and Evergreen berries processed into jam retained only 20% of the initial anthocyanins. The discrepancy in anthocyanin recovery for canned products between our results and those of Wu et al.[41] may be explained by different sampling techniques. We analyzed anthocyanins after blending the whole canned product (berries and liquid canning media), whereas Wu et al. measured anthocyanins in canned berries only. Hager et al.[40] also measured anthocyanins in the liquid and berry fractions and found that approximately 25% of the anthocyanins diffused out of the berries into the liquid canning media following processing. Patras et al.[42] compared the effect of high-pressure processing versus conventional thermal processing on total anthocyanin content of blackberry puree and found that purees subjected to three high-pressure treatments (400, 500, and 600 MPa for 15 min at 10−30 °C) incurred no anthocyanin losses and had higher levels than thermally processed puree (70 °C for 2 min), which retained 97% of the anthocyanins compared to the unprocessed sample. The excellent retention of anthocyanins observed in this study may be due to oxygen exclusion as all pureed samples were mixed and packed under vacuum prior to processing.

Extensive losses of anthocyanins occurred during processing of nonclarified and clarified juices. Following blanching, only 66% of the anthocyanins were retained, with 30% of the anthocyanins retained in the presscake, whereas the pasteurization step resulted in 35 and 23% losses of anthocyanins in nonclarified and clarified juice, respectively.[40] Gancel et al.[43] measured concentrations of cyanidin 3-glucoside and cyanidin 3-malonyl glucoside through various stages of juice processing and found that the first step of processing involving blanching, crushing, pressing, and sieving resulted in 61 and 54% retention of the two anthocyanins. The final stage of processing, pasteurization, homogenization, hot-filling, holding, and cooling, resulted in additional anthocyanin losses with the final juice retaining 48% of cyanidin 3-glucoside and 36% of cyanidin 3-malonyl glucoside. According to their findings 80% of the anthocyanin losses were attributed to thermal degradation and 20% of losses were due to removal of the waste materials. Concentration of blackberry juice from 8.9 to 65.0 °Brix was found to cause a minor (<5%) loss in anthocyanins.[44]

Black Raspberries. Anthocyanins were better retained in puree (63%), berries canned in water (58%), and berries

canned in syrup (49%) than in nonclarified (31%) and clarified (27%) juices.[45] In berries canned in water or syrup, approximately 35% of the anthocyanins diffused out of the berries into the liquid canning media.[45] During juice processing 16% of the anthocyanins were retained in the presscake, and only 1% was retained in the sediment following clarification, indicating about 45% of the anthocyanins were degraded during maceration, blanching, and depectinization steps.[45] The pasteurization process resulted in anthocyanin losses of 19 and 23% in nonclarified and clarified juices, respectively.[45]

Losses of Anthocyanins during Storage. *Blueberries.* Anthocyanin losses observed in processed products (juices, puree, and canned berries) over 6 months of storage at 25 °C were paralleled by increased polymeric color values.[27] The extensive loss of anthocyanins in berry products stored at ambient temperature is consistent with many studies. The storage temperature effect on degradation kinetics of anthocyanins in blueberry juice was reported by Buckow et al.[46] Using a 1.4-order reaction model, they reported half-lives of total anthocyanins to be 184.3, 35.0, and 5.1 days at 4, 25, and 40 °C, respectively.[46] Several studies have demonstrated the beneficial effect of refrigerated storage on reducing the rate of anthocyanin degradation in blueberry juices.[37,47] The aglycone structure and type of conjugated sugar attached influences the stability of anthocyanins during storage. Trost et al.[48] measured anthocyanins in blueberry−aronia nectar over 207 days of storage and found the ranking order of the stability of aglycones present as the glycoside from most to least stable to be Cyd > Pnd > Ptd > Mvd = Dpd. In regard to conjugated sugars, the ranking order was glucose > galactose > arabinose. They suggested that the presence of hydroxyl radical on the 3′-position improved stability compared to methoxyl radical on the same position and that substituents on the 5′-position decreased stability. The greater stability of glucose and galactose as opposed to arabinose was proposed to be due to steric hindrance as a result of the larger hexose sugars.

Blackberries. Similar to blueberry products, anthocyanins were readily degraded over 6 months of storage in blackberry products (juices, purees, and canned berries), and these changes were accompanied by increased polymeric color values.[40] The anthocyanin losses incurred during storage in purees and canned berries were much greater than losses incurred during processing. The storage temperature effect on degradation kinetics of anthocyanins in blackberry juice and concentrate was reported by Wang and Xu.[44] Using a first-order reaction model they reported half-lives at 5, 25, and 35 °C to be 330.1, 32.1, and 11.7 h for juices and 133.3, 19.0, and 7.7 h for 65 °Brix concentrate, respectively. The greater susceptibility of anthocyanins to degradation in concentrate may be due to the concentration and closer proximity of reactants such as oxygen and metals. These conditions may also promote the formation of PPs. The retentions of anthocyanins in berries canned in water (32%) or syrup (30%) after 6 months of storage at 25 °C[40] were slightly lower than retention values reported for Marion and Evergreen berries canned in water (79 and 43%) or syrup (58 and 47%) after 6 months of storage.[41] After 6 months of storage, approximately 30% of the anthocyanins had leached out of the berries into the water or syrup.[40]

Black Raspberries. Anthocyanins were readily degraded during storage of processed black raspberry products, with clarified and nonclarified juices retaining only 8 and 13% after 6 months of storage at 25 °C, whereas purees and canned berries retained 22−25%.[45] In canned berries approximately 36% of the anthocyanins diffused out of the berries into the water or syrup over 6 months of storage.[45] Similar to results observed for processed blueberry and blackberry products, anthocyanin losses during storage were accompanied by increased polymeric color values.

Polymeric Color Formation during Processing and Storage. Polymeric color is a term that has been recognized in the wine industries for some time. Timberlake et al.[49] noted desirable effects of anthocyanins on wine quality with no negative effects of the presence of polymeric color. Kalbasi and Cisneros-Zevallos[50] demonstrated a relationship between monomeric anthocyanin content and light and chroma color properties in Concord grape juice. More recent studies have been conducted evaluating the effects of processing of blueberries, blackberries, and black raspberries on the formation of percent polymeric color.[27,40,45] The method for determining percent polymeric color is based upon the subtractive procedure of Gusti and Wrolstad,[51] by which the color density of an unbleached sample and the polymeric color of a matching sample bleached with sodium bisulfite are determined with the following formulas:

$$\text{color density} = [(A_{420\text{nm}} - A_{700\text{nm}}) + (A_{512\text{nm}} - A_{700\text{nm}})]$$

$$\text{polymeric color} = [(A_{420\text{nm}} - A_{700\text{nm}}) + (A_{512\text{nm}} - A_{700\text{nm}})]$$

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

This assay is based on the principle that monomeric anthocyanins react with sodium bisulfite to form a colorless sulfonic acid adduct at C4 on the pyran ring, whereas polymeric pigments are resistant to bleaching due to attachment of tannins to the anthocyanin molecule at C4. The measurements at 420 and 700 nm correct for sample browning and haze, respectively. Hence, the ratio between polymeric color and color density determines the percentage of color contributed by PPs. Percent polymeric color values increased when berries were processed into various products, indicating that some anthocyanins may react with tannins to form PPs.[27,40,45] Blueberries and blackberries canned in syrup had very high percent polymeric color values compared to other processed products, suggesting a potential role of furfural and 5-(hydroxymethyl)furfuraldehyde (HMF) derived from heated sugars in the formation of anthocyanin−tannin polymers. Brownmiller et al.[27] tracked changes in percent polymeric color during blueberry juice processing and found that the largest increase occurred when frozen berries (0.6%) were blanched (8.6%), whereas small increases were observed in nonclarified and clarified juices following pasteurization, from 10.2 to 12.1% and from 6.2 to 7.5%, respectively. The presscake and sediment had high percent polymeric color values, 30.1 and 12.1%, respectively, indicating that the polymers were most likely formed during the depectinization step performed at 40 °C for 1 h and that the polymers have a high affinity to bind to cell wall polysaccharides/proteins. Lee et al.[33] also reported an increase in percent polymeric color following pasteurization of blueberry juice, from 37.6% for frozen fruit to 50.4% for pasteurized juice, and found that blanching and SO_2 treatments reduced percent polymeric color values of pasteurized juices,

40.7 and 42.1%, respectively, compared to the control, 50.4%. In their study percent polymeric color values increased from 50.4 to 54.3% when pasteurized juice was concentrated, but no increases in percent polymeric color occurred during concentration when berries were blanched or treated with SO_2.

Many of the changes in percent polymeric color with processing of berries were expected. However, data on freeze-dried berry powders were not available, so we analyzed several berry powders from commercial sources for the amount of polymeric color (Table 1). We also analyzed some commercial

Table 1. Anthocyanin Concentrations and Polymeric Color of Commercial Berry Juices and Freeze-Dried (FD) Berry Powders

sample	ID	anthocyanin concentration mg/g (mL)	polymeric color (%)
chokeberry juice concentrate	MSB2008	24.29	18.2
chokeberry juice concentrate	MSB2009	12.57	31.9
chokeberry FD powder l.5%	FC1195N450	12.43	43.0
blueberry FD powder	FCOOONll	30.29	20.5
wild blueberry FD powder	Std-ref	44.36	27.9
wild blueberry FD powder	OFD	27.00	17.8
blueberry FD powder	FC30582	40.56	20.9
blueberry FD powder	FC32068	36.26	22.9
blueberry anthocyanins[a]	ACNC	nd[e]	35.4
blueberry juice, stored open[b]	VD2	0.306	62.3
blueberry juice, stored NL[c]	VD3	0.447	53.9
blueberry juice, stored 4 °C[d]	VDl	1.609	35.2
bilberry FD powder	FC1687N35	61.01	10.3
blackberry FD powder	FC95N450	7.23	48.1
black currant FD powder	FC71N451	41.7	24.0
raspberry FD powder	FC16N116	11.35	23.4
strawberry FD powder	FCOON95	6.34	53.2
cranberry FD powder	DB	nd	37.7
black raspberry FD powder	OFD	nd	14.0
elderberry FD powder	FC1724N12	nd	27.8
cherry (tart) FD powder	FC0524N217	nd	26.3
Concord grape juice	WM-122009	nd	78.4

[a]Purified blueberry anthocyanins. [b]Blueberry juice stored in brown glass bottles in light at room temperature. [c]Blueberry juice stored in brown glass bottles in the dark at room temperature. [d]Blueberry juice stored in brown glass bottles in light at room temperature. [e]nd, not determined.

products including chokeberry juice concentrate, blueberry juice, and Concord grape juice. Blueberry juice, which was stored at room temperature in the light, had the lowest anthocyanin concentrations (0.306 mg/mL) and highest percent polymeric color (62.3%) compared to juice stored refrigerated (1.61 mg/mL and 35.2%, respectively) (sample VD1) (Table 1). The sample of Concord grape juice had a very high percent polymeric color. The two chokeberry juice concentrates, which were produced in two separate years, differed considerably between years, with anthocyanin concentrations decreasing and polymeric color increasing in year 2009 compared to year 2008. It was somewhat surprising to observe the relatively high amounts of polymeric color in some of the freeze-dried berry powders, which ranged from 10.3% in a bilberry sample to 48% in a blackberry freeze-dried powder (Table 1). PPs were present in all berry freeze-dried powders tested, although at various levels. The presence of PPs in a purified preparation of anthocyanins from blueberry (Table 1; ACNC) was somewhat surprising. Additional work is needed to determine at what stage in the preparation of the freeze-dried powders the PPs were formed and if they are formed during storage at low-moisture conditions.

In analyzing some of our data from black raspberry and chokeberry processing and storage in more detail (Figure 2),

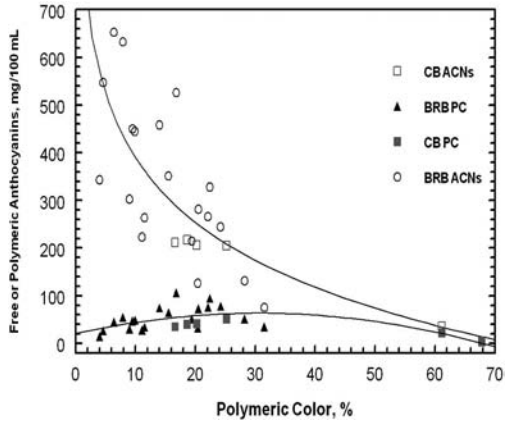

Figure 2. Free monomeric anthocyanins (ACNs) or polymeric anthocyanins (PC) from black raspberries (BRB) and chokeberry (CB) related to polymeric color (%, *X*-axis). Adapted from Hager et al.[45]

the increase in the measured percent polymeric color can be accounted for largely by the losses (degradation) of monomeric anthocyanins and not necessarily to any big increase in the amount of polymeric anthocyanins. The data on black raspberry in this figure includes effects of processing and changes during storage for up to 6 months.[45] The data for free monomeric anthocyanins was based upon HPLC analysis, and the amount of polymeric mass was calculated on the basis of the measured percent polymeric color[45] using the equation

$$\text{PACN (mg/100 mL} = ((\text{FACN, mg/100 mL})/(100 - \%\text{PC})) \times \%\text{PC}$$

where PACN is the concentration of polymeric anthocyanins (mg/100 mL), FACN is the concentration of free anthocyanins (mg/100 mL) determined by HPLC, and %PC is the percent polymeric color determined by spectrophotometric method.[51]

The data on chokeberry is based upon an accelerated storage study (unpublished data). We have calculated similar data with blueberry processing and storage, which is slightly more variable than what is presented in Figure 2, but a similar trend exists. The decline in anthocyanin content in blueberry juice and black raspberry juice with increasing polymeric color (percent) is similar (Figure 3). These results seem to suggest that monomeric anthocyanins are readily degraded during storage without any appreciable increase in polymers. The method of calculation of polymeric anthocyanin mass we used should be considered an estimate and is subject to assumptions, which may or may not hold. However, the fate of these anthocyanins is unknown as we see no evidence of the formation of chalcones or phenolic acids indicative of anthocyanin degradation in our HPLC chromatograms. The detection of chalcones by HPLC may be hindered by the low pH of the extraction solvents and/or mobile phases typically used in anthocyanin analysis. Chalcones can be converted back to flavylium cation form upon acidification, although the

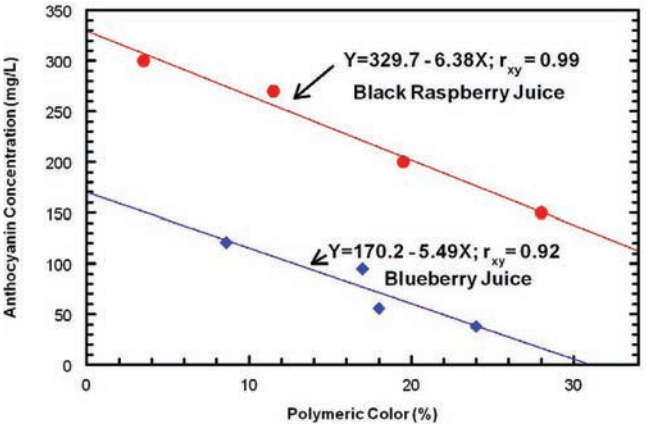

Figure 3. Relationship of monomeric anthocyanin content (mg/L) and polymeric color (%). Adapted from Brownmiller et al.[27] and Hager et al.[45]

reaction is reported to be slow, taking hours to reach completion.[52] A major limitation of the polymeric color assay is that the results indicate only the percentage of anthocyanins that is resistant to bleaching in the presence of potassium metabisulfite. The assay does not quantify the amount of PPs actually present in the sample, which is not possible due to lack of PP standards and additional information, that is, molar absorptivities and molecular weights of the standards required for quantification. Another possibility is that large molecular weight PPs form during storage to the extent they are precipitated and not recoverable for analysis. Further research is needed to address these issues.

Formation of Polymeric Pigments in Chokeberry Juice. Chokeberries were processed into nonclarified juice as previously described.[27] Following pasteurization, half of the juice was immediately placed into a 1 L plastic container and stored at −70 °C until analysis. The other half was placed into a 1 L plastic container, stored in a laboratory oven at 40 °C for 6 weeks, and then stored at −70 °C until analysis. The pasteurized chokeberry juice had a total anthocyanin content determined according to the HPLC method of Cho et al.[6] of 316 mg/100 mL, whereas the aged juice stored for 6 weeks at 40 °C contained only 67 mg/100 mL. The percent polymeric color values of the pasteurized and aged juices were 20.2 and 67.8%, respectively. The pasteurized and aged juices were purified by solid phase extraction using Sephadex LH-20 as previously described[53] and then analyzed by MALDI-TOF-MS to verify the presence of PPs and procyanidins. During MALDI-TOF-MS analysis, anthocyanins and flavan-3-ols readily associate with sodium (+22.9 amu) $[M - H + Na]^+$ and potassium (+39.1 amu) $[M - H + K]^+$ naturally present in plant extracts to form alkali metal adducts. Consistent with this phenomenon we observed three different masses for each compound reflecting the protonated molecular $[M^+]$, $[M - H + Na]^+$, and $[M - H + K]^+$ ions (Figure 4). Cyanidin 3-galactoside (m/z 449.3) is the predominant anthocyanin in chokeberries, and we detected m/z values indicative of the addition of multiple flavan-3-ol (m/z 288) units to the anthocyanin moiety in both pasteurized and aged juices (Figures 5 and 6). For simplicity, only the $[M - H + K]^+$ ions are labeled in Figures 5 and 6, and the peak assignments for mass spectral data are presented in Table 2. The results demonstrate the linkage of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 flavan-

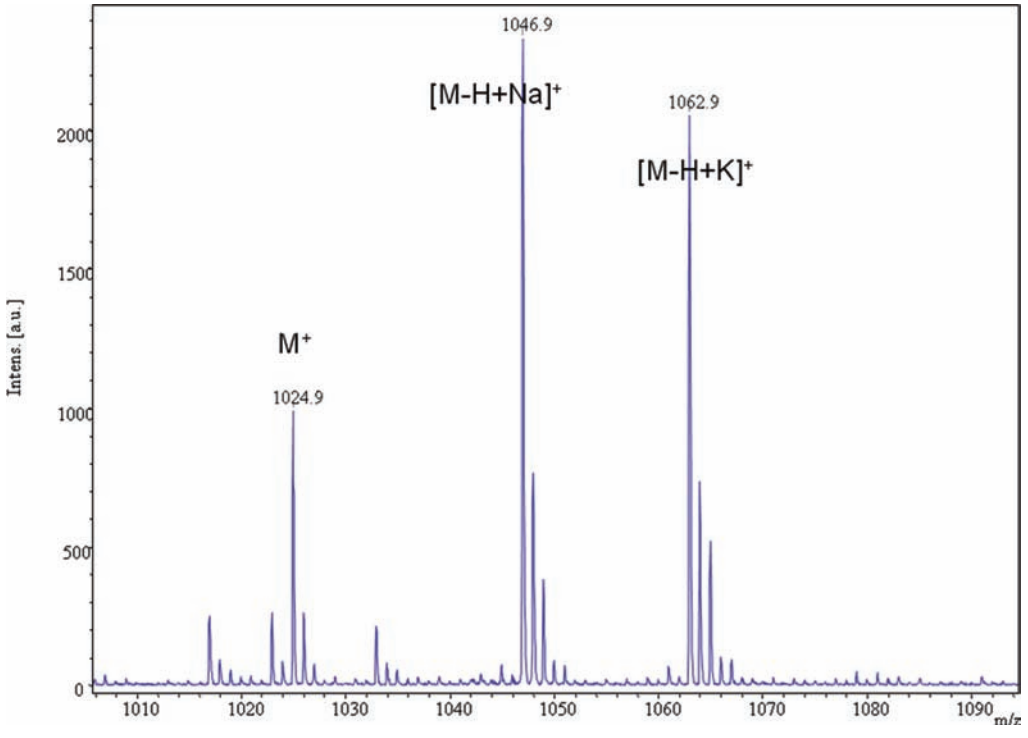

Figure 4. MALDI-TOF mass spectra of cyanidin 3-galactoside linked to 2 flavan-3-ol units demonstrating the M^+, $[M - H + Na]^+$ and $[M - H + K]^+$ forms. Following Sephadex LH-20 cleanup, procyanidin and PP-rich fractions from fresh and aged juice were mixed with a 1 M solution of dihydroxybenzoic acid in 100% methanol in a 1:7 ratio (sample/matrix), and 1 μL of the mixture was spotted onto a ground stainless steel MALDI target for MALDI analysis using the dry droplet method. MALDI analysis was performed using a Bruker Reflex III MALDI-TOF-MS (Billerica, MA) equipped with a N_2 laser set at 337 nm, with all data obtained in positive ion reflectron mode.

dx.doi.org/10.1021/jf2046575 | J. Agric. Food Chem. 2012, 60, 6678–6693

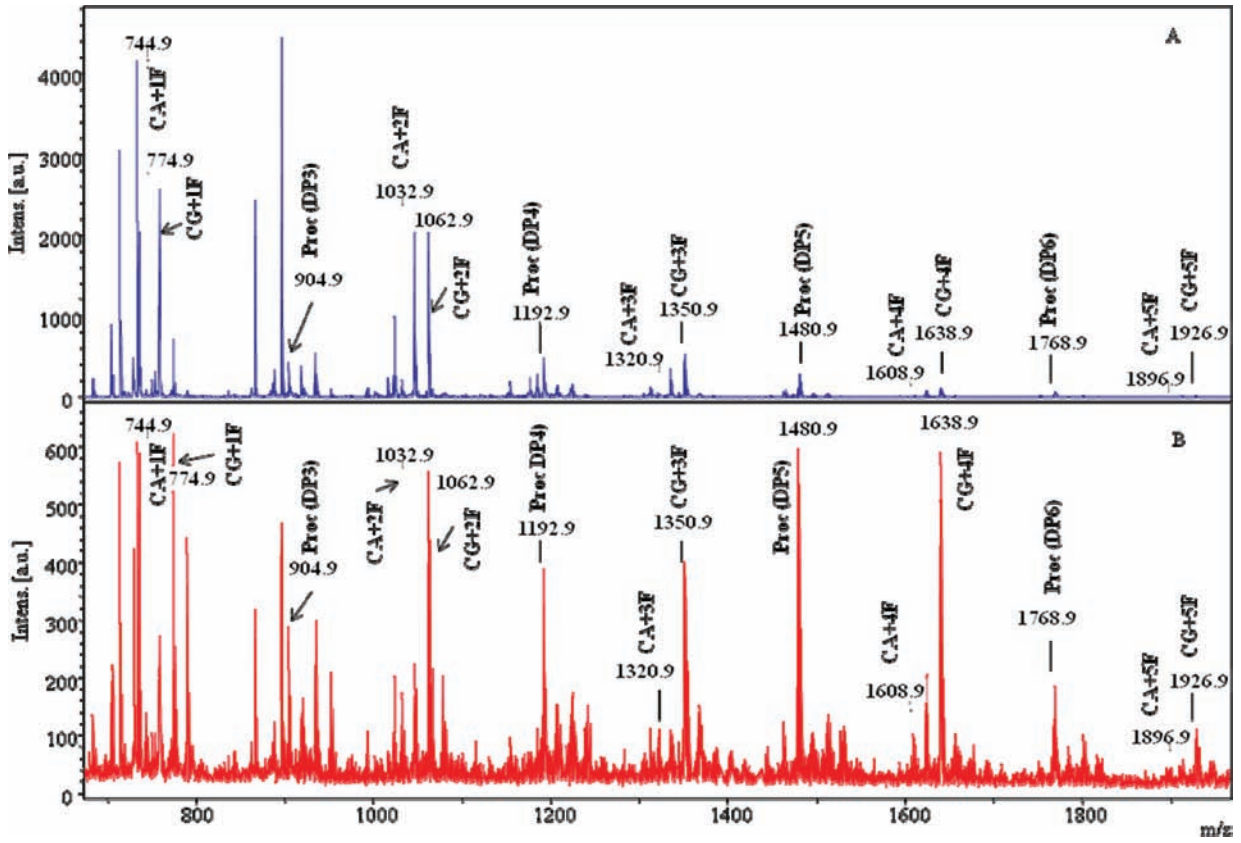

Figure 5. MALDI-TOF mass spectra (m/z 730−1875) of (A) pasteurized and (B) aged juice in reflectron mode showing the series $[M - H + K]^+$ of cyanidin 3-arabinoside plus 1 flavan-3-ol unit (m/z 744.9) to cyanidin 3-glactoside plus 5 flavan-3-ol units (m/z 1926.9) and procyanidin series ranging from DP3 (m/z 904.9) to DP6 (m/z 1768.9). CA, cyanidin 3-arabinoside; CG, cyanidin 3-galactoside; F, flavan-3-ol.

3-ol units via B-type bonds to cyanidin 3-galactoside, respectively. There was no evidence of a CH_3−CH bridge (+16 amu) linking the terminal anthocyanin unit to flavan-3-ol, indicating PPs were formed via a direct condensation reaction. The presence of procyanidins with B-type linkages of $[M - H + K]^+$ ranging from DP3 (m/z 904.9) to DP12 (m/z 3496.9) was also observed in the MALDI-TOF mass spectra of pasteurized and aged juices (Figures 5 and 6; Table 2). Our results are consistent with those of Wu et al.,[54] who found chokeberry procyanidins to range from DP 1 to DP 8. Cyanidin 3-arabinoside (m/z 419.4), the second most abundant anthocyanin in chokeberries, was also found linked to 1, 2, 3, 4, 5, 6, and 7 flavan-3-ol units via B-type bonds in both pasteurized and aged juices (Figures 5 and 6; Table 2). To ascertain differences among MALDI-TOF-MS results for pasteurized and aged juices, 10 sets of MALDI-TOF mass spectra from each were compared after normalization using Bruker Daltonics ClinPro Tools version 2.2 software. Average normalized peak intensities are shown in Figure 7. The peaks of pasteurized juice show greater signal intensities over the m/z range of 700−1450, indicating a greater abundance of cyanidin 3-galactoside and cyanidin 3-arabinoside linked to 1, 2, and 3, flavan-3-ol units and procyanidin dimers, trimers, and tetramers compared to aged juice (Figure 7A). However, the peaks of aged juice show greater signal intensities over the m/z range of 1450−3000, indicating a greater abundance of cyanidin 3-galactoside and cyanidin 3-arabinoside linked to more than flavan-3-ol units and procyanidin oligomers with DP ≥5 compared to pasteurized juice (Figure 7B). The normalized peaks areas of PPs identified in pasteurized and aged juices are presented in Table 3. The pasteurized juice contained greater amounts of cyanidin 3-galactoside linked to 1, 2, and 3 flavan-3-ols units and cyanidin 3-arabinoside linked to 2 flavan-3-ol units than the aged juice, whereas the aged juice contained greater amounts of cyanidin 3-galactoside linked to 4, 5, 6, and 7 flavan-3-ol units and cyanidin 3-arabinoside linked to 4 flavan-3-ol units than the pasteurized juice. These results demonstrate that low molecular weight PPs are formed in response to pasteurization, but during storage additional flavan-3-ol units are attached to anthocyanin moieties, resulting in the formation of large molecular weight compounds. It is plausible that anthocyanins are polymerized with flavan-3-ol units to very high molecular weight compounds that eventually precipitate and form sediment in the stored juice. Additional research is needed to characterize the large molecular weight PPs that form in chokeberry juice during storage, although this will be an analytical challenge due to diminished resolution of peaks m/z >3000.

COMPOSITIONAL CHANGES AND LOSSES OF PROCYANIDINS DURING BLUEBERRY PROCESSING

Processing Losses of Blueberry Procyanidins. Extensive losses of procyanidins were observed when blueberries were processed into various products, with only 19 and 23% of the total procyanidins found in frozen berries retained in nonclarified and clarified juices and 41, 65, and 78% retained in purees, berries canned in syrup, and berries canned in water, respectively.[53] As observed for anthocyanins, the larger number and complexity of processing steps involved in juicing resulted

dx.doi.org/10.1021/jf2046575 | J. Agric. Food Chem. 2012, 60, 6678−6693

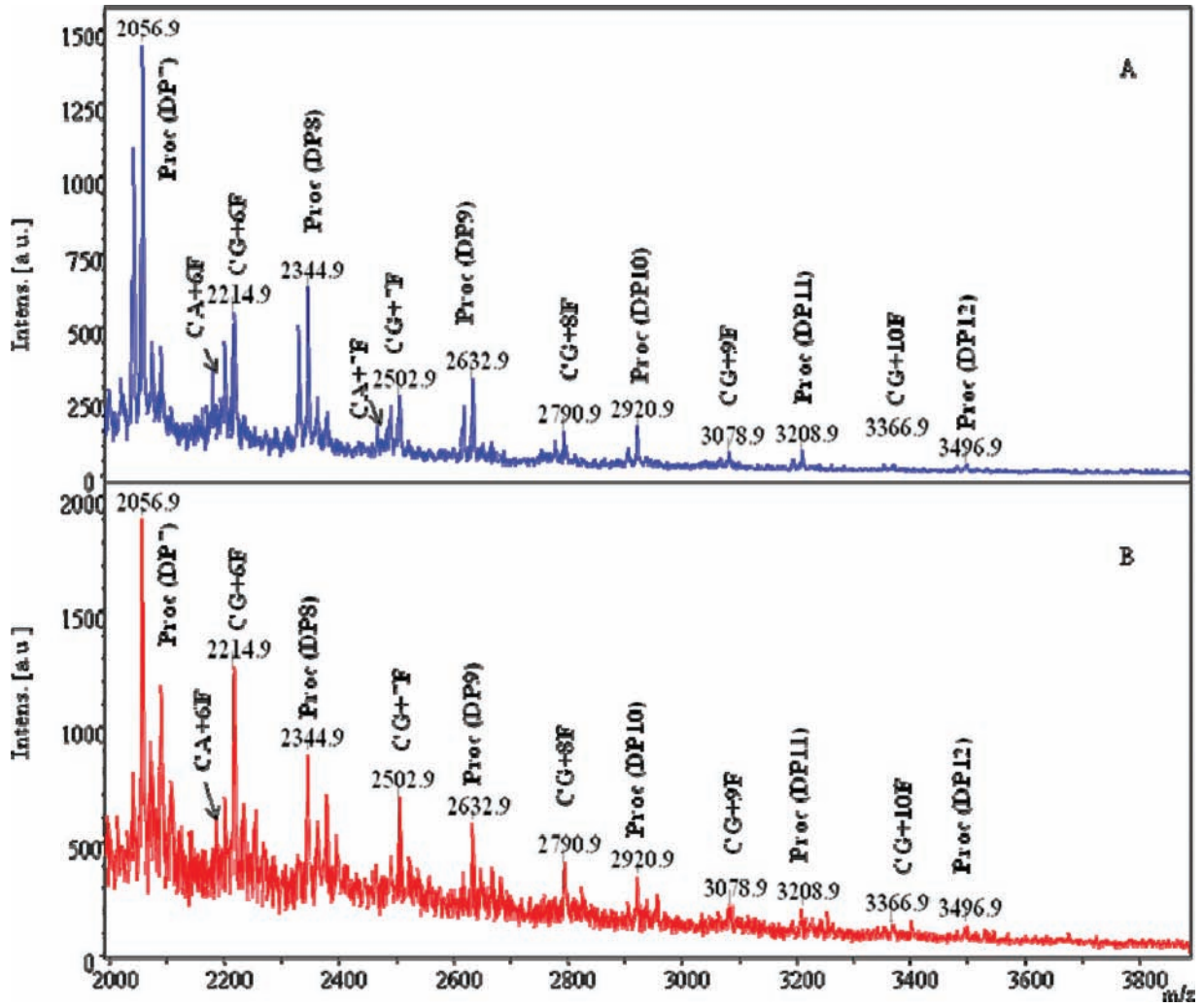

Figure 6. MALDI-TOF mass spectra (m/z 2000−3850) of (A) pasteurized and (B) aged juice in reflectron mode showing the series $[M - H + K]^+$ of cyanidin 3-galactoside plus 6 flavan-3-ol units (m/z 2214.9) to cyanidin 3-galactoside plus 10 flavan-3-ol units (m/z 3366.9) and procyanidin series ranging from DP7 (m/z 2056.9) to DP12 (m/z 3496.9). CA, cyanidin 3-arabinoside; CG, cyanidin 3-galactoside; F, flavan-3-ol.

in the greatest losses of procyanidins.[53] Following blanching, 79% of the total procyanidins were retained, whereas only 47 and 36% were retained in the nonclarified and clarified juices following depectinization and pressing treatments.[53] A significant amount of procyanidins (24%) was retained in the presscake due to the physical separation of the procyanidin-rich seeds, and 10% of the procyanidins were found in the sediment following clarification.[53] In contrast to anthocyanins, pasteurization resulted in severe losses of procyanidins, 60 and 37%, in nonclarified and clarified juices, respectively.[53] Interestingly, the simple canning process, which involves placing thawed berries into the can, covering the berries with liquid canning medium (syrup or water), and pasteurization, resulted in the greatest retention of procyanidins. This is most likely the result of limited enzymatic degradation as the berries remain intact during processing.

Generally, procyanidin oligomers having a DP of >3 were lost more readily than monomers and dimers, which was evident following blanching and continued throughout processing.[53] In nonclarified pasteurized juice 41 and 50% of monomers and dimers were retained, whereas <10% of trimers through hepatmers were retained and octamers were nondetectable.[53] In clarified pasteurized juice, 38 and 58% of monomers and dimers were retained, 24 and 20% of trimers and tetramers were retained, <11% of pentamers through heptamers were retained, and octamers were nondetectable. The 38% retention of monomers in clarified juice was similar to values (25−44% retention) reported in other studies for clarified blueberry juice.[32,37] The greater retention of monomers and dimers throughout processing indicates that low molecular weight procyanidins bind less strongly to cell wall polysaccharides/proteins[55,56] and/or are more resistant to thermal degradation.

Similar to juice processing, monomers and dimers were better retained than higher oligomers in purees and canned blueberries during processing.[53] This trend was consistent with the higher polymeric color value observed for berries canned in syrup and may reflect HMF- and furfural-induced formation of anthocyanin−tannin polymers.[53] The greater losses of larger oligomers in purees and canned berries were consistent with changes observed following blanching in juice processing, which involved heating and mashing of the berries. This was most likely a result of preferential binding of larger oligomers to cell polysaccharides/proteins, but it is also possible that larger oligomers were depolymerized to monomers and dimers in response to thermal treatment.

dx.doi.org/10.1021/jf2046575 | J. Agric. Food Chem. 2012, 60, 6678−6693

Table 2. MALDI-TOF-MS Peak Assignments

compound	m/z (±200 ppm)
cyd 3-ara + 1 flavan-3-ol	744.9
cyd 3-gal + 1 flavan-3-ol	774.9
procyanidin trimer	904.9
cyd 3-ara + 2 flavan-3-ols	1032.9
cyd 3-gal + 2 flavan-3-ols	1062.9
procyanidin tetramer	1192.9
cyd 3-ara + 3 flavan-3-ols	1320.9
cyd 3-gal + 3 flavan-3-ols	1350.9
procyanidin pentamer	1480.9
cyd 3-ara + 4 flavan-3-ols	1608.9
cyd 3-gal + 4 flavan-3-ols	1638.9
procyanidin hexamer	1768.9
cyd 3-ara + 5 flavan-3-ols	1896.9
cyd 3-gal + 5 flavan-3-ols	1926.9
procyanidin heptamer	2056.9
cyd 3-ara + 6 flavan-3-ols	2184.9
cyd 3-gal + 6 flavan-3-ols	2214.9
procyanidin octamer	2344.9
cyd 3-ara + 7 flavan-3-ols	2472.9
cyd 3-gal + 7 flavan-3-ols	2502.9
procyanidin nonamer	2632.9
cyd 3-gal + 8 flavan-3-ols	2790.9
procyanidin decamer	2920.9
cyd 3-gal + 9 flavan-3-ols	3078.9
procyanidin 11-mer	3208.9
cyd 3-gal + 10 flavan-3-ols	3366.9
procyanidin 12-mer	3496.9

Storage Losses of Blueberry Procyanidins. Levels of total procyanidins declined markedly during 6 months of storage of blueberry products at 25 °C, with berries canned in water or syrup retaining the highest amounts, 38 and 29%, respectively, whereas juices and purees retained <11%.[53] Similar to changes observed in response to processing, monomers and dimers in all processed products were found to be more stable during storage than larger oligomer.[53]

LOSSES AND COMPOSITIONAL CHANGES OF ELLAGITANNINS DURING BLACKBERRY AND BLACK RASPBERRY PROCESSING AND STORAGE

Processing Losses of Ellagitannins. *Blackberries.* The effect of processing blackberries into juices, purees, and canned products on total ellagitannins was reported by Hager et al.[57] Ellagitannins were well retained in purees and berries canned in water or syrup, but only 18 and 30% were retained in clarified and nonclarified juices, respectively. The juice processing steps had various effects on ellagitannins, with a 43% increase observed following blanching, presumably as a result of seed softening by the steam treatment and enhanced extraction.[57] The greatest loss of ellagitannins occurred during the pressing operation, when 67% of the ellagitannins were retained in the presscake.[57] Blackberry ellagitannins are located predominantly in seeds,[7,58] and their exclusion by pressing largely accounts for the low recoveries in nonclarified (31%) and clarified (12%) juices. Consistent with the excellent recoveries in pureed and canned products, ellagitannin levels were not affected by pasteurization, indicating they are heat stable.[57] Blackberry ellagitannins were also reported to be stable during jam processing.[59]

According to Hager et al.,[57] the ellagitannin composition of blackberries changes little in response to processing. Castalagin/vescalagin and pedunculagin isomers increased modestly in canned and pureed products, which may be due to depolymerization of larger molecular weight compounds with processing or enhanced extraction due to thermal treatment of the seeds. Gancel et al.[43] monitored changes in lambertianin C, sanguiin H6, and ellagic acid during processing of blackberry juice. They found that 80% of lambertianin C and sanguiin H6 was retained after the initial blanching, crushing, pressing, and sieving step, with some hydrolysis to ellagic acid observed. Significant losses were also observed during the final processing step, which involved pasteurization, homogenization, hot-filling, holding, and cooling, resulting in only 20 and 50% retentions of lambertianin C and sanguiin H6 in the final juice. They concluded that 66 and 58% of the losses of the two ellagitannins were the result of thermal degradation with the remaining losses attributed to removal in the waste streams.

Black Raspberries. Hager[25] studied the effect of processing black raspberries into juices, purees, and canned products on total ellagitannins. Similar to blackberries, levels of total ellagitannins were well retained in purees and berries canned in water or syrup, but only 31 and 33% were retained in clarified and nonclarified juices, respectively.[25] In black raspberries canned in water or syrup approximately 23% of the ellagitannins leached out of the berries into the liquid canning media. The major losses of ellagitannins during juice processing occurred mainly following blanching and maceration (39% loss) and pressing (33% loss), whereas the compounds were not affected by pasteurization.[25]

Storage Losses of Ellagitannins in Blackberry Products. *Blackberries.* Total ellagitannins were well retained in purees and berries canned in water over 6 months of storage at 25 °C, but losses were evident in nonclarified and clarified juices and berries canned in syrup.[57] The major losses in nonclarified juices and berries canned in syrup were mainly due to losses in large molecular weight ellagitannins, which appeared to be depolymerized to ellagic acid.[57] In canned blackberries approximately 10−20% of the ellagitannins leached out of the berries into the liquid canning media during storage.[57]

Black Raspberries. Total ellagitannins were well retained in all processed black raspberry products over 6 months of storage at 25 °C, with apparent increases observed in clarified juices and berries canned in syrup.[25] Although total ellagitannin levels changed little during storage, levels of ellagic acid increased in all processed products during storage with the exception of purees, indicating hydrolysis of large molecular weight ellagitannins.[25] In canned black raspberries approximately 20−30% of the ellagitannins leached out of the berries into the liquid canning media during storage.[25]

MECHANISMS RESPONSIBLE FOR ANTHOCYANIN AND PROCYANIDIN LOSSES DURING PROCESSING AND STORAGE

The stability of anthocyanins is influenced by many factors including chemical structure, pH, temperature, enzymes, oxygen, light, and the presence of ascorbic acid, sugars, metals, and copigments.[60,61] Stability increases with increasing number of methoxyl groups and attached sugars. Acylation of anthocyanins with cinnamic and organic acids greatly improves color stability by preventing hydrolysis of the red flavylium cation form, resulting in the formation of blue quinoidal bases,

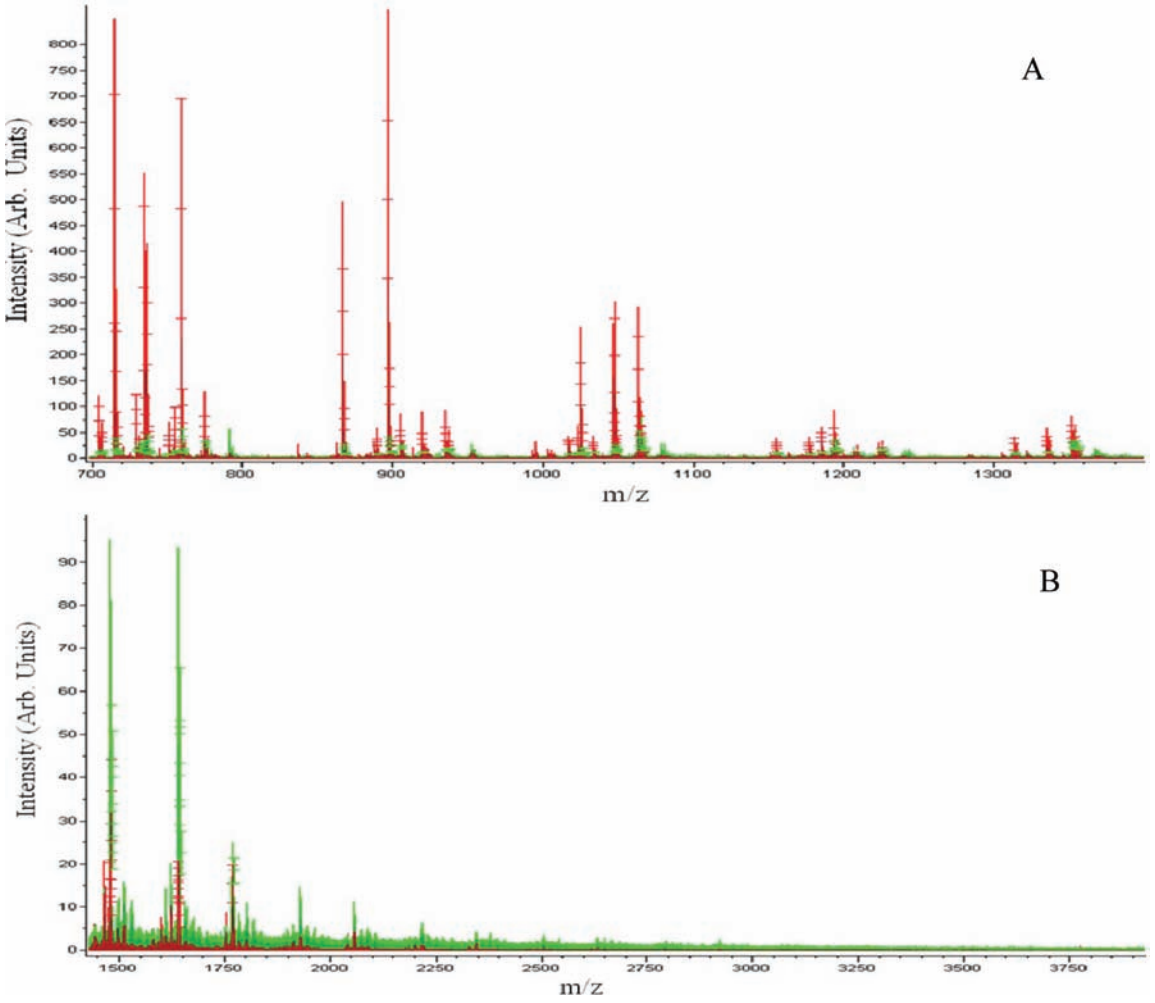

Figure 7. Normalized MALDI-TOF-MS peak areas of polymeric pigments from pasteurized (red) and aged (green) chokeberry juices over m/z ranges of (A) 700−1400 and (B) 1450−3800.

which show greater color stability at higher pH values.[61] Additionally, acylated anthocyanins are more resistant to degradation by heat, light, and SO_2. The pH of foods, especially berry products, has a marked effect on anthocyanin retention and color stability. Under extreme acidic conditions (pH ≤2), the red-colored flavylium cation form predominates, but in the pH range of most berry juices (pH 2−4), rapid hydration of the flavylium cation occurs, resulting in the formation of the colorless carbinol pseudobase.[61] The carbinol pseudobase can be converted slowly over time to an open ring structure, the chalcone pseudobase.[61] Consistent with this mechanism, blueberry juice acidified to pH 1 was shown to have higher levels of monomeric anthocyanins and lower polymeric color values than juices adjusted to pH 4 and 7.[26] A linear relationship exists between the rate of anthocyanin degradation and processing temperature, which is best described by first-order reaction kinetics.[44,46] It has been proposed that thermal degradation of anthocyanins at pH 3.5, which is typical of many berry products, involves opening of the pyrilium ring and formation of a chalcone glycoside, followed by cleavage of the glycoside to form a chalcone, which spontaneously degrades into phenolic acids (4-hydroxybenzoic acid, protocatechuic acid) and phloroglucinaldehyde.[62]

Enzymes, namely, polyphenol oxidase (PPO) and peroxidase (POD), can also cause rapid degradation of anthocyanins prior to thermal inactivation and possibly during storage if they are not totally inactivated through pasteurization. PPO and POD have been shown to degrade anthocyanins in the presence of cofactors such as chlorogenic acid for PPO[29,30] and chlorogenic acid and H_2O_2 for POD.[31] The chlorogenoquinones formed via these reactions react with anthocyanins to form colorless compounds, which can further react to form brown melanin polymers. Skrede et al.[32] provided indirect evidence for enzymatic degradation of anthocyanins in a study on blueberry juice, in which the addition of fresh blueberry extract to pasteurized juice resulted in significant loss of anthocyanins, whereas addition of a blanched extract caused no loss. Enzymes may also play an important role in anthocyanin degradation during storage of processed berry products if they are not inactivated by thermal treatments. In a study on strawberry nectar, both extended pasteurization time and addition of enzyme inhibitor improved color stability during storage, suggesting residual enzyme activity plays an important role in color degradation.[63]

Additionally, exposure of fruit products to oxygen during processing and storage can have a detrimental effect on anthocyanins and color stability, especially in the presence of high levels of ascorbic acid and metals.[60] Kalt et al.[26] reported that blueberry juice samples stored in vials that were completely filled and had limited oxygen-containing headspace experienced

dx.doi.org/10.1021/jf2046575 | J. Agric. Food Chem. 2012, 60, 6678−6693

Table 3. Normalized MALDI-TOF-MS Peak Areas of Polymeric Pigments in Pasteurized and Aged Chokeberry Juices

compound	pasteurized peak area	aged peak area
procyanidin dimer	21.7 ± 8.4[a]	10.9 ± 3.8
cyd 3-ara + 1 flavan-3-ol	1.7 ± 0.4	1.2 ± 0.1
cyd 3-gal + 1 flavan-3-ol	9.4 ± 2.9	4.4 ± 0.9
procyanidin trimer	54.9 ± 7.5	8.0 ± 0.5
cyd 3-ara + 2 flavan-3-ols	11.7 ± 0.8	4.4 ± 0.2
cyd 3-gal + 2 flavan-3-ols	78.8 ± 9.2	11.7 ± 1.2
procyanidin tetramer	14.5 ± 1.3	7.2 ± 0.6
cyd 3-ara + 3 flavan-3-ols	3.2 ± 0.3	3.5 ± 0.1
cyd 3-gal + 3 flavan-3-ols	17.5 ± 1.6	7.7 ± 0.6
procyanidin pentamer	1.7 ± 0.4	5.5 ± 1.3
cyd 3-ara + 4 flavan-3-ols	0.5 ± 0.2	1.6 ± 0.2
cyd 3-gal + 4 flavan-3-ols	5.3 ± 0.5	11.2 ± 1.4
procyanidin hexamer	3.2 ± 0.5	3.9 ± 0.3
cyd 3-ara + 5 flavan-3-ols	0.2 ± 0.1	0.7 ± 0.1
cyd 3-gal + 5 flavan-3-ols	1.0 ± 0.3	2.7 ± 0.2
procyanidin heptamer	1.2 ± 0.3	2.6 ± 0.2
cyd 3-ara + 6 flavan-3-ols	0.1 ± 0.1	0.5 ± 0.1
cyd 3-gal + 6 flavan-3-ols	0.2 ± 0.2	1.0 ± 0.1
procyanidin octamer	0.3 ± 0.2	0.9 ± 0.1
cyd 3-ara + 7 flavan-3-ols	<0.1 ± <0.1	0.3 ± 0.1
cyd 3-gal + 7 flavan-3-ols	0.1 ± 0.1	0.7 ± 0.2
procyanidin nonamer	0.1 ± 0.2	0.6 ± 0.2
cyd 3-gal + 8 flavan-3-ols	<0.1 ± 0.1	0.3 ± 0.1
procyanidin decamer	<0.1 ± <0.1	0.3 ± 0.1
cyd 3-gal + 9 flavan-3-ols	≪0.1 ± ≪0.1	0.2 ± ≪0.1
procyanidin 11-mer	≪0.1 ± ≪0.1	0.2 ± ≪0.1
cyd 3-gal + 10 flavan-3-ols	≪0.1 ± ≪0.1	0.1 ± ≪0.1
procyanidin 12-mer	≪0.1 ± ≪0.1	0.1 ± ≪0.1

[a]Total of M^+, $(M - H + Na)^+$, and $(M - H + K)^+$ peak areas were included depending on the detection level. Standard deviations listed are based on 10 spectra from each sample (n = 10).

no anthocyanin loss over 6 h, whereas samples with 50% headspace lost 76% of monomeric anthocyanins. Anthocyanins in blackberry puree were well retained following pasteurization when the berries were mixed and packed under vacuum.[42] Pomegranate juice and blueberry–aronia nectars packed in glass showed greater color stability and retention of anthocyanins than products stored in carton packages.[48,64] Strawberry puree and juice prepared under nitrogen retained higher levels of anthocyanins than samples prepared under air, but nitrogen did not affect the degradation of anthocyanins or the formation of PPs in the products during storage at 20 °C.[65] Exposure of anthocyanins to UV and visible light can also cause degradation and increase the rate of thermal degradation,[60] but light exposure does not appear to play as great a role as oxygen as noted in the studies on pomegranate juice and blueberry–aronia nectars, in which products packed in opaque cartons retained fewer anthocyanins and less color than products packed in glass.

Studies on processed berry products consistently show that marked losses of anthocyanins and procyanidins during processing, and especially during storage, are accompanied by increased polymeric color values, indicative of the formation of PPs. It is well-known that PPs form during storage of red wines, altering the color from bright red to a reddish brown tint, and various mechanisms have been postulated to explain the formation of pigments responsible for color change. The first mechanism involves a reaction between anthocyanins and flavanols mediated by a variety of compounds including acetaldehyde,[66−68] glyoxylic acid,[67] and furfural and 5-(hydroxymethyl)furfuraldehyde (HMF).[69] The second mechanism involves a direct condensation of anthocyanins with flavanols.[70−74] Direct condensation reactions between anthocyanins and ellagitannins may occur in ellagitannin-rich berries as malvidin and malvidin glucoside were shown to complex with vescalagin in a model system.[75] Although most anthocyanin–procyanidin polymers have been identified in simple model systems involving single anthocyanin glycosides and catechin, polymers up to DP 8 (octamer) composed of an anthocyanin combined with a procyanidin heptamer formed via a direct condensation reaction were identified in red wine.[76] Similarly, polymers up to DP 6 (hexamer) composed of an anthocyanin combined with a procyanidin pentamer formed via an acetaldehyde condensation reaction (ethyl linkages between anthocyanins and flavan-3-ols) have been identified in spray-dried cranberry juice.[77] Complex polymers proposed to consist of anthocyanins and procyanidins have also been isolated from Concord grape juice and were found to have a molecular weight around 12000 Da.[78]

Anthocyanins can also react with free hydroxycinnamic acids (coumaric, caffeic, ferulic, sinapic) as well as pyruvic acid and acetaldehyde to form pyranoanthocyanins. These novel compounds have been identified in strawberry and raspberry juices.[79] However, in our previous studies on blueberry, blackberry, and black raspberry juices we did not observe any new peaks in our HPLC traces of samples stored for 6 months indicative of pyranoanthocyanin formation.

Another potential mechanism that may explain the marked losses of procyanidins during storage of blueberry juice[53] involves binding of the large molecular weight procyanidins to cell wall polymers. Procyanidins, especially those of high DP, show a great propensity to bind to cell wall polysaccharides through hydrogen bonding and/or hydrophobic interactions.[55,56] It is plausible that procyanidins, and possibly PPs, bind to polysaccharide or protein colloids in juice and eventually precipitate during long-term storage. Ellagitannins in processed blackberry products[57] appear to be much more stable during storage than procyanidins in processed blueberry products,[53] but it is also possible that they bind to polysaccharide or protein colloids and form a precipitate during storage. Ellagitannin and ellagitannin–protein complexes are reported to contribute to haze and sediment formation in blackberry juice.[80]

POTENTIAL METHODS/TREATMENTS TO MITIGATE ANTHOCYANIN AND PROCYANIDIN LOSSES DURING PROCESSING AND STORAGE

The extensive losses of anthocyanins in berry juices during processing appear to be mainly attributable to the action of oxidoreductases and physical removal of epidermal tissue during pressing, whereas losses of tannins, procyanidins in blueberries, and ellagitannins in blackberries and black raspberries are mainly due to physical removal of seeds during pressing. Methods are needed to rapidly inactivate oxidoreductases in frozen berries typically used for processing. Potential thermal treatments include pulsed electric fields (PEF) or microwave processing or nonthermal treatments such as high hydrostatic pressure (HHP) and ultrasonics, possibly coupled with thermal treatment. The use of ultrasonics, HHP, and PEF coupled with heat treatment at 70 °C has been shown

to improve the extraction of anthocyanins from grape byproducts, with PEF showing greater efficacy to extract anthocyanin monoglucosides and HHP greater ability to extract acylated anthocyanins.[81] Alternatively, strict oxygen exclusion during mashing and pressing through the use of inert gases such as nitrogen or carbon dioxide may prevent enzyme activity and allow extraction to be performed in the absence of heat. The addition of natural inhibitors of PPO and POD could also be useful in controlling polyphenol losses during the initial stages of processing and possibly during storage if the enzymes are not totally inactivated by blanching and pasteurization. Because waste materials, especially presscake, retain appreciable levels of polyphenols, methods are needed to facilitate their extraction into the juice. Further advancements in enzyme preparations used in mashing will continue to improve juice yields and extraction of polyphenols, but more detailed information on cell wall composition of berries is needed to tailor the application of cell wall hydrolases, esterases, and proteases. An alternative approach would be to extract polyphenols from waste materials and add them back to the juice. Van der Sluis et al.[82] showed it was possible to increase the polyphenol content of apple juice by extracting polyphenols from pomace with ethanol, evaporating the ethanol under vacuum to 50−60 °Brix, diluting the concentrate to 12 °Brix, and then adding the extract to the conventional juice. Pressurized hot water extraction of polyphenols from waste materials might be an alternative to the use of ethanolic extraction due to environmental and tariff issues associated with the use of ethanol in foods. Pressurized hot water has been shown to be an effective technology for extracting anthocyanins from a variety of berry waste materials,[83,84] but methods are needed to concentrate the aqueous extracts without further application of heat. Although pasteurization of berry juices needed to ensure saftety (5 log pathogen reduction) generally results in minor (<12%) losses of anthocyanins, further work is needed to determine the times and temperatures needed to inactivate PPO and POD. Strawberry PPO was recently found to be highly resistant to both thermal and high-pressure inactivation, whereas POD was much less thermostable.[85] The presence of thermostable PPO and/or POD in other berries could lead to extensive losses of anthocyanins and other polyphenols during processing as well as long-term storage at ambient temperature.

Dense phase carbon dioxide (DPCD) is a promising technology for processing anthocyanin-rich berry juices. DPCD was shown to result in complete retention of anthocyanins in muscadine grape juice compared to a 16% loss in thermally processed juice, and the DPCD-processed juice retained high levels of anthocyanins over 10 weeks of storage at 4 °C.[86] The greater stability of anthocyanins in DPCD-treated juice was attributed to reduced oxidation through displacement of dissolved oxygen and inactivation of PPO.

In canned berries, appreciable levels of polyphenols diffuse out of the berries into the liquid canning media in response to processing and during storage. This is influenced by the anatomical characteristics of the berry epidermal tissue, that is, the presence of natural wax barriers, the number and size of pores for liquid migration, and the structural integrity of the berry. Because leaching of polyphenols, especially anthocyanins, is difficult to control due to their solubility, consumers should realize that they will not receive the same levels of polyphenols by exclusion of the liquid canning media.

It is clear that anthocyanins undergo condensation reactions with procyanidins during storage of berry products to form PPs. Unfortunately, these PPs have not been well characterized, so we know little about their constituents, linkages, or molecular weights. There are also limitations associated with the polymeric color assay as some pigments resistant to sulfite bleaching, for example, pyranoanthocyanins, which have a substituent on the C-ring, are not polymeric, and conversely some PPs that do not have a substituent on the C-ring readily bleach in the presence of sulfite. Potential strategies to prevent the formation of PPs may entail addition of food grade chemicals to block the site on the C-ring (carbon 4) where procyanidins most commonly attach. Addition of SO_2 ameliorated the loss of anthocyanins in strawberry puree and juice during storage at 20 °C and decreased the rate of PP formation, presumably due to the presence of the sulfonic acid adduct on C4, which blocks the condensation reaction.[65] Encapsulation of monomeric anthocyanins with cyclodextrins or other encapsulation agents may prove to be useful if they can engulf the whole anthocyanin molecule or at least the C-ring. Improved thermal stability of anthocyanins isolated from *Hibiscus sabdariffa* in the presence of β-cyclodextrin was reported by Mourtzinos et al.,[87] despite limited inclusion (B-ring only) of anthocyanins in the β-cyclodextrin cavity. The addition of phenolic acids to juices may also block the formation of PPs through their ability to act as intramolecular copigments. Rein et al.[88] reported that addition of cinnamic acids to berry juices enhanced juice color, and they observed novel HPLC peaks indicative of intramolecular copigmentation compounds.

Challenges exist to develop effective and cost-effective treatments to retain monomeric anthocyanins in processed berry products. At present, refrigerated storage is the most effective strategy available to maintain color and polyphenol content in berry products. The use of packaging materials with excellent oxygen barrier properties also seems to be beneficial in retaining anthocyanins during storage.

POTENTIAL IMPLICATIONS OF PROCESSING- AND STORAGE-INDUCED CHANGES ON BIOACTIVE PROPERTIES ASSOCIATED WITH ANTHOCYANINS AND PROCYANIDINS

Many of the health benefits associated with berry consumption are thought to be due to anthocyanins, procyanidins, and ellagitannins. Therefore, the extensive losses of these compounds incurred in response to processing and storage of berries would be expected to have an adverse effect on bioactive properties and health outcomes. Few studies have investigated the effects of processing and storage of processed berry products on bioactive properties. In a study involving a wide variety of commercial blueberry products, polyphenol fractions isolated from thermally treated products (cooked, canned, jam, juice, spray-dried, syrups, pie fillings) were found to have lower antiproliferation activities than fractions isolated from non-thermally treated samples including fresh, individually quick-frozen (IQF), and freeze-dried berries.[89] In another study antiproliferation activity of blueberry juice was shown to decline in response to storage time, which coincided with marked losses of anthocyanins over storage.[37]

Unfortunately, little is known about the bioavailability of PPs, although in a recent study a polymeric pigment fraction isolated from acai showed limited absorption in a Caco-2 intestinal cell

model and significantly decreased transport of monomeric anthocyanins.[90] Although absorption of the PPs may be limited, they may undergo transformation by intestinal microflora, resulting in the production of phenolic acids (PA), which are much smaller and simpler in structure than the PPs. The presence of large amounts of monophenolic acids has been demonstrated previously in the colon of healthy humans.[91] Increased absorption of phenolic acids has been observed when sorghum bran (high in procyanidins)[92] or a procyanidin-rich extract from cranberries[93] was fed to rats. Although no data are available, conversion of PPs is likely to occur in the large intestine with PAs being absorbed into the circulation, which may have beneficial health-promoting effects. Previous research has demonstrated that some PAs may have positive health effects. Ferulic acid (4-hydroxy-3-methoxycinnamic acid) has been shown to reduce hypertension,[94] lipid peroxidation,[95] and oxidative impairments[96] and to enhance insulin secretion in rats, whereas *p*-methoxycinnamic acid has been found to stimulate insulin secretion from pancreatic β-cells in rats.[97] Caffeic acid has been shown to improve glucose utilization and reduce plasma glucose in diabetic rats,[98] whereas chlorogenic acid attenuates hypertension and endothelial cell function in spontaneously hypertensive rats.[94] Similarly, *o*-coumaric acid was found to be effective in improving the symptoms of metabolic syndrome and obesity.[99,100] 3,4-Dihydroxybenzoic acid (protocatechuic acid), found in many edible and medicinal plants and a major metabolite of cyanidin-3-glucoside, has been shown to have chemopreventive effects[101−103] and to improve antioxidant status,[104] a major factor involved in many chronic diseases. However, the extent and importance of the absorption of many of the PAs is not known primarily because of the difficulty in assessing all of the breakdown products of the parent polyphenols.

Feeding of blackberry puree containing seeds resulted in an increased plasma antioxidant capacity for a period of 8 h following the meal, which was not observed at 8 h with blackberry juice, suggesting that ellagitannins or other larger molecular weight polyphenols were metabolized by microflora in the lower gut into compounds that could be absorbed and contribute to the increased antioxidant capacity.[58] If PPs are degraded in a similar manner in the lower gut into phenolic acids, the absorption of these compounds from the colon may still impart health benefits.

AUTHOR INFORMATION

Corresponding Author

*Phone: (479) 575-2978. Fax: (479) 575-6936. E-mail: lukeh@uark.edu..

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Seeram, N. P. Berry fruits: compositional elements, biochemical activities, and the impact of their intake on human health, performance, and disease. *J. Agric. Food Chem.* **2008**, *56*, 627−629.

(2) Seeram, N. P. Recent trends and advances in berry health benefits research. *J. Agric. Food Chem.* **2010**, *58*, 3869−3870.

(3) Gao, L.; Mazza, G. Quantitation and distribution of simple and acylated anthocyanins and other phenolics in blueberries. *J. Food Sci.* **1994**, *59*, 1057−1059.

(4) Prior, R. L.; Lazarus, S. A.; Cao, G.; Muccitelli, H.; Hammerstone, J. F. Identification of procyanidins and anthocyanins in blueberries and cranberries (*Vaccinium* spp.) using high-performance liquid chromatography/mass spectrometry. *J. Agric. Food Chem.* **2001**, *49*, 1270−1276.

(5) Wu, X.; Prior, R. L. Systematic identification and characterization of anthocyanins by HPLC-ESI-MS/MS in common foods in the United States: fruits and berries. *J. Agric. Food Chem.* **2005**, *53*, 2589−2599.

(6) Cho, M. J.; Howard, L. R.; Prior, R. L.; Clark, J. R. Flavonoid glycosides and antioxidant capacity of various blackberry, blueberry, and red grape genotypes determined by high-performance liquid chromatography/mass spectrometry. *J. Sci. Food Agric.* **2004**, *84*, 1771−1782.

(7) Siriwoharn, T.; Wrolstad, R. E.; Finn, C. E.; Pereira, C. B. Influence of cultivar, maturity, and sampling on blackberry (*Rubus* L. hybrids) anthocyanins, polyphenolics, and antioxidant properties. *J. Agric. Food Chem.* **2004**, *52*, 8021−8030.

(8) Wu, X.; Pittman, H. E.; McKay, S.; Prior, R. L. Aglycones and sugar moieties alter anthocyanin absorption and metabolism following berry consumption in the weanling pig. *J. Nutr.* **2005**, *135*, 2417−2424.

(9) Tian, Q.; Giusti, M. M.; Stoner, G. D.; Schwartz, S. J. Characterization of a new anthocyanin in black raspberries (*Rubus occidentalis*) by liquid chromatography electrospray ionization tandem mass spectrometry. *Food Chem.* **2006**, *94*, 465−468.

(10) Roghani, M.; Niknam, A.; Jalali-Nadoushan, M. R.; Kiasalari, Z.; Khalili, M.; Baluchnejadmojarad, T. Oral pelargonidin exerts dose-dependent neuroprotection in 6-hydroxydopamine rat model of hemiparkinsonism. *Brain Res. Bull.* **2010**, *82*, 279−283.

(11) Takikawa, M.; Inoue, S.; Horio, F.; Tsuda, T. Dietary anthocyanin-rich bilberry extract ameliorates hyperglycemia and insulin sensitivity via activation of AMP-activated protein kinase in diabetic mice. *J. Nutr.* **2010**, *140*, 527−533.

(12) Prior, R. L.; Wilkes, S. E.; Rogers, T. R.; Khanal, R. C.; Wu, X.; Howard, L. R. Purified blueberry anthocyanins and blueberry juice alter development of obesity in mice fed an obesogenic high-fat diet. *J. Agric. Food Chem.* **2010**, *58*, 3970−3976.

(13) Mauray, A.; Felgines, C.; Morand, C.; Mazur, A.; Scalbert, A.; Milenkovic, D. Bilberry anthocyanin-rich extract alters expression of genes related to atherosclerosis development in aorta of apo E-deficient mice. *Nutr. Metab. Cardiovasc. Dis.* **2010**, DOI: 1016/j.numecd.2010.04.011.

(14) Krikorian, R.; Shidler, M. D.; Nash, T. A.; Kalt, W.; Vinqvist-Tymchuk, M. R.; Shukitt-Hale, B.; Joseph, J. A. Blueberry supplementation improves memory in older adults. *J. Agric. Food Chem.* **2010**, *58*, 3996−4000.

(15) Broncel, M.; Kozirog, M.; Duchnowicz, P.; Koter-Michalak, M.; Sikora, J.; Chojnowska-Jezierska, J. Aronia melanocarpa extract reduces blood pressure, serum endothelium, lipid, and oxidative stress marker levels in patients with metabolic syndrome. *Med. Sci. Monit.* **2010**, *16*, CR28−CR34.

(16) Seymour, M.; Tanone, I.; Lewis, S.; Urcuyo-Llanes, D.; Bolling, S. F.; Bennink, M. R. Blueberry-enriched diets reduce metabolic syndrome and insulin resistance in rats. *FASEB J.* **2009**, *23*, 563.31.

(17) Qin, Y.; Xia, M.; Ma, J.; Hao, Y.; Liu, J.; Mou, H.; Cao, L.; Ling, W. Anthocyanin supplementation improves serum LDL- and HDL-cholesterol concentrations associated with the inhibition of cholesteryl ester transfer protein in dyslipidemic subjects. *Am. J. Clin. Nutr.* **2009**, *90*, 485−492.

(18) DeFuria, J.; Bennett, G.; Strissel, K. J.; Perfield, J. W. 2nd; Milbury, P. E.; Greenberg, A. S.; Obin, M. S. Dietary blueberry attenuates whole-body insulin resistance in high fat-fed mice by reducing adipocyte death and its inflammatory sequelae. *J. Nutr.* **2009**, *139*, 1510−1516.

(19) Wang, L. S.; Stoner, G. D. Anthocyanins and their role in cancer prevention. *Cancer Lett.* **2008**, *269*, 281−290.

(20) Wang, D.; Zou, T.; Yang, Y.; Yan, X.; Ling, W. Cyanindin-3-*O*-β-glucoside with the aid of its metabolite protocatechuic acid, reduces monocyte infiltration in apolipoprotein E-deficient mice. *Biochem. Pharmacol.* **2011**, *82*, 713−719.

(21) Min, J.; Yu, S. W.; Baek, S. H.; Nair, K. M.; Bae, O. N.; Bhatt, A.; Kassab, M.; Nair, M. G.; Majid, A. Neutroprotective effect of cyanidin-3-*O*-glucoside anthocyanin in mice with focal cerebral ischemia. *Neurosci. Lett.* **2011**, *500*, 157−161.
(22) Gu, L.; Kelm, M. A.; Hammerstone, J. F.; Beecher, G.; Holden, J.; Haytowitz, D.; Gebhardt, S.; Prior, R. L. Concentrations of proanthocyanidins in common foods and estimations of normal consumption. *J. Nutr.* **2004**, *134*, 613−617.
(23) Gasperotti, M.; Masuero, D.; Vrhovsek, U.; Guella, G.; Mattivi, F. Profiling and accurate quantification of Rubus ellagitannins and ellagic acid conjugates using direct UPLC-Q-TOF HDMS and HPLC-DAD analysis. *J. Agric. Food Chem.* **2010**, *58*, 4602−4616.
(24) Hager, T. J.; Howard, L. R.; Liyanage, R.; Lay, J. O.; Prior, R. L. Ellagitannin composition of blackberry as determined by HPLC-ESI-MS and MALDI-TOF-MS. *J. Agric. Food Chem.* **2008**, *56*, 661−669.
(25) Hager, A. C. *Processing and storage effects on black raspberry polyphenolics and antioxidant capacity*. Thesis, University of Arkansas, Fayetteville, 2008.
(26) Kalt, W.; McDonald, J. E.; Donner, H. Anthocyanins, phenolics, and antioxidant capacity of processed lowbush blueberry products. *J. Food Sci.* **2000**, *65*, 390−393.
(27) Brownmiller, C.; Howard, L. R.; Prior, R. L. Processing and storage effects on monomeric anthocyanins, percent polymeric color, and antioxidant capacity of processed blueberry products. *J. Food Sci.* **2008**, *73*, H72−H79.
(28) Sablani, S. S.; Andrews, P. K.; Davies, N. M.; Walters, T.; Saez, H.; Syamaladevi, R. M.; Mohekar, P. R. Effect of thermal treatments on phytochemicals in conventionally and organically grown berries. *J. Sci. Food Agric.* **2010**, *90*, 769−779.
(29) Kader, F.; Rovel, B.; Girardin, M.; Metche, M. Mechanism of browning in fresh highbush blueberry fruit (*Vaccinium corymbosum* L.). Partial purification and characterization of blueberry polyphenol oxidase. *J. Sci. Food Agric.* **1997**, *73*, 513−516.
(30) Kader, F.; Rovel, B.; Girardin, M.; Metche, M. Mechanism of browning in fresh highbush blueberry fruit (*Vaccinium corymbosum* L.). Role of blueberry polyphenol oxidase, chlorogenic acid and anthocyanins. *J. Sci. Food Agric.* **1997**, *74*, 31−34.
(31) Kader, F.; Irmouli, M.; Nicolas, J. P.; Metche, M. Involvement of blueberry peroxidase in the mechanisms of anthocyanin degradation in blueberry juice. *J. Food Sci.* **2002**, *67*, 910−915.
(32) Skrede, G.; Wrolstad, R. E.; Durst, R. W. Changes in anthocyanins and polyphenolics during juice processing of highbush blueberries (*Vaccinium corymbosum* L.). *J. Food Sci.* **2000**, *65*, 357−364.
(33) Lee, J.; Durst, R. W.; Wrolstad, R. E. Impact of juice processing on blueberry anthocyanins and polyphenolics: comparison of two pretreatments. *J. Food Sci.* **2002**, *67*, 1660−1667.
(34) Rossi, M.; Giussani, E.; Morelli, R.; Lo Scalzo, R.; Nani, R. C.; Torreggiani, D. Effect of fruit blanching on phenolics and radical scavenging activity of highbush blueberry juice. *Food Res. Int.* **2003**, *36*, 999−1005.
(35) Buchert, J.; Koponen, J. M.; Suutarinen, M.; Mustranta, A.; Lille, M.; Torronen, R.; Poutanen, K. Effect of enzyme-aided pressing on anthocyanin yield and profiles in bilberry and blackcurrent juices. *J. Sci. Food Agric.* **2005**, *85*, 2548−2556.
(36) Kechinski, C. P.; Guimarães, P. V. R.; Noreña, C. P. Z.; Tessaro, I. C.; Marczak, L. D. F. Degradation kinetics of anthocyanin in blueberry juice during thermal treatment. *J. Food Sci.* **2010**, *75*, C173−C176.
(37) Srivastava, A.; Akoh, C. C.; Yi, W.; Fischer, J.; Krewer, G. Effect of storage conditions on the biological activity of phenolic compounds of blueberry packed in glass bottles. *J. Agric. Food Chem.* **2007**, *55*, 2705−2713.
(38) Brambilla, A.; Lo Scalzo, R.; Bertolo, G.; Torreggiani, D. Steam-blanched highbush blueberry (*Vaccinium corymbosum* L.) juice: phenolic profile and antioxidant capacity in relation to cultivar selection. *J. Agric. Food Chem.* **2008**, *56*, 2643−2648.
(39) Ichiyanagi, T.; Oikawa, K.; Tateyama, C.; Konishi, T. Acid mediated hydrolysis of blueberry anthocyanins. *Chem. Pharm. Bull.* **2001**, *49*, 114−123.
(40) Hager, T. J.; Howard, L. R.; Prior, R. L. Processing and storage effects on monomeric anthocyanins, percent polymeric color, and antioxidant capacity of processed blackberry products. *J. Agric. Food Chem.* **2008**, *56*, 689−695.
(41) Wu, R.; Frei, B.; Kennedy, J. A.; Zhao, Y. Effects of refrigerated storage and processing technologies on the bioactive compounds and antioxidant capacities of 'Marion' and 'Evergreen' blackberries. *LWT − Food Sci. Technol.* **2010**, *43*, 1253−1264.
(42) Patras, A.; Brunton, N. P.; Da Pieve, S.; Butler, F. Impact of high pressure processing on total antioxidant activity, phenolic, ascorbic acid, anthocyanin content and colour of strawberry and blackberry purees. *Innovative Food Sci. Emerging Technol.* **2009**, 308−313.
(43) Gancel, A. L.; Feneuil, A.; Acosta, O.; Perez, A. M.; Vaillant, F. Impact of industrial processing and storage on major polyphenols and the antioxidant capacity of tropical highland blackberry (*Rubus adenotrichus*). *Food Res. Int.* **2011**, *44*, 2243−2251.
(44) Wang, W. D.; Xu, S. Y. Degradation kinetics of anthocyanins in blackberry juice and concentrate. *J. Food Eng.* **2007**, *82*, 271−275.
(45) Hager, A.; Howard, L. R.; Prior, R. L.; Brownmiller, C. Processing and storage effects on monomeric anthocyanins, percent polymeric color, and antioxidant capacity of processed black raspberry products. *J. Food Sci.* **2008**, *73*, H134−H140.
(46) Buckow, R.; Kastell, A.; Shiferaw, T.; Versteeg, C. Pressure and temperature effects on degradation kinetics and storage stability of total anthocyanins in blueberry juice. *J. Agric. Food Chem.* **2010**, *58*, 10076−10084.
(47) Simard, R. E.; Bourzeix, M.; Heredia, N. Factors influencing color degradation in blueberry juice. *Lebensm. Wissen. Technol.* **1982**, *15*, 177−180.
(48) Trošt, K.; Golic-Wondra, M.; Prošek, M.; Milivojevič, L. Anthocyanin degradation of blueberry-aronia nectar in glass compared with carton during storage. *J. Food Sci.* **2008**, *73*, S405−S411.
(49) Timberlake, C. F.; Bridle, P.; Jackson, M. G.; Vallis, L. Correlations between quality and pigment parameters in young Beaujolais red wines. *Ann. Nutr. Aliment.* **1978**, *32*, 1095−1101.
(50) Kalbasi, A.; Cisneros-Zevallos, L. Fractionation of monomeric and polymeric anthocyanins from Concord grape (*Vitis labrusca* L.) juice by membrane ultrafiltration. *J. Agric. Food Chem.* **2007**, *55*, 7036−7042.
(51) Giusti, M. M.; Wrolstad, R. E. Characterization and measurement of anthocyanins with UV-visible spectroscopy. In *Current Protocols in Food Analytical Chemistry*; Wrolstad, R. E., Ed.; Wiley: New York, 2001; pp F1.2.1−F1.2.13.
(52) Francis, F. J. Food colorants: anthocyanins. *Crit. Rev. Food Sci. Nutr.* **1989**, *28*, 273−241.
(53) Brownmiller, C. R.; Howard, L. R.; Prior, R. L. Processing and storage effects on procyanidin composition and concentration of processed blueberry products. *J. Agric. Food Chem.* **2009**, *57*, 1896−1902.
(54) Wu, X.; Gu, L.; Prior, R. L.; McKay, S. Characterization of anthocyanins and proanthocyanidins in some cultivars of *Ribes*, *Aronia*, and *Sambucus* and their antioxidant capacity. *J. Agric. Food Chem.* **2004**, *52*, 7846−7856.
(55) Renard, C. M. G. C.; Baron, A.; Guyot, S.; Drilleau, J. F. Interaction between apple cells walls and native apple polyphenols: quantification and some consequences. *Int. J. Biol. Macromol.* **2001**, *29*, 115−125.
(56) Le Bourvellec, C.; Guyot, S.; Renard, C. M. G. C. Non-covalent interaction between procyanidins and apple cell wall material. Part 1. Effect of some environmental parameters. *Biochim. Biophys. Acta* **2004**, *1672*, 192−202.
(57) Hager, T. J.; Howard, L. R.; Prior, R. L. Processing and storage effects on the ellagitannin composition of processed blackberry products. *J. Agric. Food Chem.* **2010**, *58*, 11749−11754.

(58) Hager, T. J. *Processing effects on the antioxidant capacity, polyphenolic content, absorption and metabolism of Apache blackberries.* Dissertation, University of Arkansas, Fayetteville, 2007.
(59) Amakura, Y.; Umino, Y.; Tsuji, S.; Tonogai, Y. Influence of jam processing on the radical scavenging activity and phenolic content in berries. *J. Agric. Food Chem.* **2000**, *48*, 6292−6297.
(60) Jackman, R. L.; Yada, R. Y.; Tung, M. A.; Speers, R. A. Anthocyanins as food colorants − a review. *J. Food Biochem.* **1987**, *11*, 201−247.
(61) Patras, A.; Brunton, N. P.; O'Donnell, C.; Tiwari, B. K. Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. *Trends Food Sci. Technol.* **2010**, *21*, 3−8.
(62) Sadilova, E.; Carle, R.; Stintzing, F. C. Thermal degradation of anthocyanins and its impact on color and *in vitro* antioxidant capacity. *Mol. Nutr. Food Res.* **2007**, *51*, 1461−1471.
(63) Gössinger, M.; Ullram, T.; Hermes, M.; Wendelin, S.; Berghold, S.; Halbwirth, H.; Stich, K.; Berghofer, E. Effects of pre-freezing, puree content and pasteurization regime on colour stability of strawberry nectar made from puree. *J. Sci. Food Agric.* **2009**, *89*, 144−149.
(64) Pérez-Vicente, A.; Serrano, P.; Abellán, P.; Garcia-Viguera, C. Influence of packaging material on pomegranate juice colour and bioactive compounds during storage. *J. Sci. Food Agric.* **2004**, *84*, 639−644.
(65) Bakker, J.; Bridle, P. Strawberry juice colour: the effect of sulfur dioxide and EDTA on the stability of anthocyanins. *J. Sci. Food Agric.* **1992**, *60*, 477−481.
(66) Timberlake, C. F.; Bridle, P. Interactions between anthocyanins, phenolic compounds, and acetaldehyde and their significance in red wines. *Am. J. Enol. Vitic.* **1976**, *27*, 97−105.
(67) Dallas, C.; Ricardo-da-Silva, J. M.; Laureano, O. Products formed in model wine solutions involving anthocyanins, procyanidin B2 and acetaldehyde. *J. Agric. Food Chem.* **1996**, *44*, 2402−2407.
(68) Es-Safi, N. E.; Fulcrand, H.; Cheyneir, V.; Moutounet, M. Studies on the acetaldehyde-induced condensation of (−)-epicatechin and malvidin-3-*O*-glucoside in a model solution system. *J. Agric. Food Chem.* **1999**, *47*, 2096−2102.
(69) Es-Safi, N. E.; Cheyneir, V.; Moutounet, M. Interactions between cyanidin 3-*O*-glucoside and furfural derivatives and their impact on food color changes. *J. Agric. Food Chem.* **2002**, *50*, 5586−5595.
(70) Somers, T. C. The polymeric nature of wine pigments. *Phytochemistry* **1971**, *10*, 2175−2186.
(71) Jurd, L. Review of polyphenol condensation reactions and their possible occurrence in the aging of wines. *Am. J. Enol. Vitic.* **1969**, *20*, 191−195.
(72) Liao, H.; Cai, Y.; Haslam, E. Polyphenol interactions. Anthocyanins: co-pigmentation and color changes in red wines. *J. Sci. Food Agric.* **1992**, *59*, 299−305.
(73) Remy, S.; Fulcrand, H.; Labarbe, B.; Cheyneir, V.; Moutounet, M. First confirmation in red wine of products resulting from direct anthocyanin-tannin reactions. *J. Sci. Food Agric.* **2000**, *80*, 745−751.
(74) Remy-Tanneau, S.; Guerneve, C. L.; Meudec, E.; Cheyneir, V. Characterization of a colorless anthocyanin-flavan-3-ol dimer containing both carbon−carbon and ether interflavanoid linkages by NMR and mass spectrometries. *J. Agric. Food Chem.* **2003**, *51*, 3592−3597.
(75) Quideau, S.; Jourdes, M.; Lefeuvre, D.; Montaudon, D.; Saucier, C.; Glories, Y.; Pardon, P.; Pourquier, P. The chemistry of wine polyphenolic *C*-glycosidic ellagitannins targeting human topoisomerase II. *Chem.−Eur. J.* **2005**, *11*, 6503−6513.
(76) Hayasaka, Y.; Kennedy, J. A. Mass spectrometric evidence for the formation of pigmented polymers in red wine. *Aust. J. Grape Wine Res.* **2003**, *9*, 210−220.
(77) Reed, J. D.; Krueger, C. G.; Vestling, M. M. MALDI-TOF mass spectrometry of oligomeric food polyphenols. *Phytochemistry* **2005**, *66*, 2248−2263.
(78) Lin, R. I.; Hilton, B. W. Purification of commercial grape pigment. *J. Food Sci.* **1980**, *45*, 297−306.
(79) Rein, M. J.; Ollilainen, V.; Vahermo, M.; Yli-Kauhaluoma, J.; Heinonen, M. Identification of novel pyranoanthocyanins in berry juices. *Eur. Food Res. Technol.* **2005**, *220*, 239−244.
(80) Siriwoharn, T.; Wrolstad, R. E.; Durst, R. W. Identification of ellagic acid in blackberry juice sediment. *J. Food Sci.* **2005**, *70*, C189−C208.
(81) Corrales, M.; Topefl, S.; Butz, P.; Knorr, D.; Tauscher, B. Extraction of anthocyanins from grape by-products assisted by ultrasonics, high hydrostatic pressure or pulsed electric fields: a comparison. *Innovative Food Sci. Emerging Technol.* **2008**, *9*, 85−91.
(82) Van Der Sluis, A. A.; Dekker, M.; Skrede, G.; Jongen, W. M. F. Activity and concentration of polyphenolic antioxidants in apple juice. 2. Effect of novel production methods. *J. Agric. Food Chem.* **2004**, *52*, 2840−2849.
(83) King, J. W.; Gabriel, R. D.; Wightman, J. D. Subcritical water extraction of anthocyanins from fruit berry substrates. *Proceedings of the 6th International Symposium on Supercritical Fluids*, Tome I, Versailles, France, 2003; pp 409−509
(84) Ju, Z. Y.; Howard, L. R. Effects of solvent and temperature on pressurized liquid extraction of anthocyanins and total phenolics from dried red grape skin. *J. Agric. Food Chem.* **2003**, *51*, 5207−5213.
(85) Terefe, N. S.; Yang, Y. H.; Knoerzer, K.; Buckow, R.; Versteeg, C. High pressure and thermal inactivation kinetics of polyphenol oxidase and peroxidase in strawberry puree. *Innovative Food Sci. Emerging Technol.* **2010**, *11*, 52−60.
(86) Del Pozo-Insfan, D.; De Follo-Martinez, A.; Talcott, S. T.; Brenes, C. H. Stability of copigmented anthocyanins and ascorbic in muscadine grape juice processed by high hydrostatic pressure. *J. Food Sci.* **2007**, *72*, S247−S253.
(87) Mourtzinos, I.; Makris, D. P.; Yannakopoulou, K.; Kalogeropoulos, N.; Michali, I.; Karathanos, V. T. Thermal stability of anthocyanin extract of *Hibiscus sabdariffa* L. in the presence of *β*-cyclodextrin. *J. Agric. Food Chem.* **2008**, *56*, 10303−10310.
(88) Rein, M. J.; Heinonen, M. Stability and enhancement of berry juice color. *J. Agric. Food Chem.* **2004**, *52*, 3106−3114.
(89) Schmidt, B. M.; Erdman, J. W. Jr.; Lila, M. A. Effects of food processing on blueberry antiproliferation and antioxidant activity. *J. Food Sci.* **2005**, *70*, S389−S394.
(90) Pacheco-Palencia, L. A.; Mertens-Talcott, S. U.; Talcott, S. T. *In vitro* absorption and antiproliferative activities of monomeric and polymeric anthocyanin fractions from acai fruit (*Euterpe oleracea* Mart.). *Food Chem.* **2010**, *119*, 1071−1078.
(91) Jenner, A. M.; Rafter, J.; Halliwell, B. Human fecal water content of phenolics: the extent of colonic exposure to aromatic compounds. *Free Radical Biol. Med.* **2005**, *38*, 763−772.
(92) Gu, L.; House, S. E.; Rooney, L.; Prior, R. L. Sorghum bran in the diet dose dependently increased the excretion of catechins and microbial-derived phenolic acids in female rats. *J. Agric. Food Chem.* **2007**, *55*, 5326−5334.
(93) Prior, R. L.; Rogers, T. R.; Khanal, R. C.; Wilkes, S. E.; Wu, X.; Howard, L. R. Urinary excretion of phenolic acids in rats fed cranberry. *J. Agric. Food Chem.* **2010**, *58*, 3940−3949.
(94) Suzuki, A.; Yamamoto, N.; Jokura, H.; Yamamoto, M.; Fujii, A.; Tokimitsu, I.; Saito, I. Chlorogenic acid attenuates hypertension and improves endothelial function in spontaneously hypertensive rats. *J. Hypertens.* **2006**, *24*, 1075−1082.
(95) Balasubashini, M. S.; Rukkumani, R.; Viswanathan, P.; Menon, V. P. Ferulic acid alleviates lipid peroxidation in diabetic rats. *Phytother. Res.* **2004**, *18*, 310−314.
(96) Thyagaraju, B. M. Muralidhara Ferulic acid supplements abrogate oxidative impairments in liver and testis in the streptozotocin-diabetic rat. *Zoolog. Sci.* **2008**, *25*, 854−860.
(97) Adisakwattana, S.; Moonsan, P.; Yibchok-Anun, S. Insulin-releasing properties of a series of cinnamic acid derivatives in vitro and in vivo. *J. Agric. Food Chem.* **2008**, *56*, 7838−7844.
(98) Hsu, F. L.; Chen, Y. C.; Cheng, J. T. Caffeic acid as active principle from the fruit of *Xanthium strumarium* to lower plasma glucose in diabetic rats. *Planta Med.* **2000**, *66*, 228−230.

(99) Hsu, C. L.; Wu, C. H.; Huang, S. L.; Yen, G. C. Phenolic compounds rutin and o-coumaric acid ameliorate obesity induced by high-fat diet in rats. *J. Agric. Food Chem.* **2009**, *57*, 425−431.
(100) Hsu, C. L.; Yen, G. C. Effects of flavonoids and phenolic acids on the inhibition of adipogenesis in 3T3-L1 adipocytes. *J. Agric. Food Chem.* **2007**, *55*, 8404−8410.
(101) Liu, Y. M.; Jiang, B.; Bao, Y. M.; An, L. J. Protocatechuic acid inhibits apoptosis by mitochondrial dysfunction in rotenone-induced PC12 cells. *Toxicol. In Vitro* **2008**, *22*, 430−437.
(102) Lin, H. H.; Chen, J. H.; Huang, C. C.; Wang, C. J. Apoptotic effect of 3,4-dihydroxybenzoic acid on human gastric carcinoma cells involving JNK/p38 MAPK signaling activation. *Int. J. Cancer* **2007**, *120*, 2306−2316.
(103) Krajka-Kuźniak, V.; Kaczmarek, J.; Baer-Dubowska, W. Effect of naturally occurring phenolic acids on the expression of glutathione S-transferase isozymes in the rat. *Food Chem. Toxicol.* **2008**, *46*, 1097−1102.
(104) Vitaglione, P.; Donnarumma, G.; Napolitano, A.; Galvano, F.; Gallo, A.; Scalfi, L.; Fogliano, V. Protocatechuic acid is the major human metabolite of cyanidin-glucosides. *J. Nutr.* **2007**, *137*, 2043−2048.