DOI:10.1021/jf902829g

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Proximate and Polyphenolic Characterization of Cranberry Pomace†

Brittany L. White,[§] Luke R. Howard,*[,§] and Ronald L. Prior[#]

[§]Department of Food Science, University of Arkansas, 2650 North Young Avenue, Fayetteville, Arkansas 72703 and [#]USDA−ARS Arkansas Children's Nutrition Center, 1120 Marshall Street, Little Rock, Arkansas 72202

The proximate composition and identification and quantification of polyphenolic compounds in dried cranberry pomace were determined. Proximate analysis was conducted based on AOAC methods for moisture, protein, fat, dietary fiber, and ash. Other carbohydrates were determined by the difference method. Polyphenolic compounds were identified and quantified by HPLC-ESI-MS. The composition of dried cranberry pomace was 4.5% moisture, 2.2% protein, 12.0% fat, 65.5% insoluble fiber, 5.7% soluble fiber, 8.4% other carbohydrates, 1.1% ash, and 0.6% total polyphenolics. It contained six anthocyanins (111.5 mg/100 g of DW) including derivatives of cyanidin and peonidin. Thirteen flavonols were identified (358.4 mg/100 g of DW), and the aglycones myricetin (55.6 mg/100 g of DW) and quercetin (146.2 mg/100 g of DW) were the most prominent. Procyanidins with degrees of polymerization (DP) of 1−6 were identified (167.3 mg/100 g of DW), the most abundant being an A-type of DP2 (82.6 mg/100 g of DW).

KEYWORDS: Anthocyanins; cranberry; flavonoids; flavonols; polyphenolics; pomace; procyanidins

INTRODUCTION

Cranberry pomace is the main byproduct of the cranberry-processing industry. It is composed primarily of skin, seeds, and stems left over after pressing the fruit for juice or preparing it for canning. Cranberry pomace has limited applications in animal feeds because of its low protein content, and it presents environmental problems when it is disposed of in the soil or landfills because of its low pH (*1*). It is important to the cranberry-processing industry that alternate uses of cranberry waste material be evaluated.

Fruits, including cranberries, are often touted for their health benefits (*2*). Historically, cranberry juice has been consumed to prevent urinary tract infections. These and other health benefits, including reduced risks of cancer and cardiovascular disease, are believed to be due to the presence of various polyphenolic compounds, including anthocyanins, flavonols, and procyanidins (*3*, *4*). Given that the seeds and skins of fruits are known to contain significant levels of polyphenolics, cranberry pomace, which includes seeds and skins, should be explored as a source of these compounds.

Anthocyanins are pink, blue, red, or purple pigments that are present primarily in epidermal tissues of fruits and vegetables (*5*). They may be present in different forms based on pH, which, in turn, determines whether they are colored or uncolored. They are generally glycosylated with various sugars that increase their stability. The aglycone forms of anthocyanins, known as anthocyanidins, are very unstable. There are six major anthocyanidins naturally present, and the two commonly found in cranberries, cyanidin and peonidin, are presented in **Figure 1a**,**b**.

Flavonols are a class of flavonoids that are characterized by a hydroxyl group at position 3, a carbonyl group at position 4, and a double bond between carbons 2 and 3 on the C ring of the flavan structure. Quercetin and myricetin are the two flavonols commonly found in cranberries (**Figure 1c**,**d**). Like anthocyanins, flavonols are typically glycosylated. They may also be acylated with phenolic acids (*6*). Unlike anthocyanins, flavonols are typically colorless, but they absorb light strongly in the UV range and appear yellow at high concentrations. Cranberries contain particularly high levels of derivatives of the flavonol quercetin compared to other fruits.

Procyanidins are a class of polymeric flavonoids composed of flavan-3-ol units, primarily epicatechin, which may be converted to cyanidin upon heating with acid (*7*). Epicatechin monomers, in turn, may be linked together by one of two options. The most common linkages between the flavan rings are the $4\beta \rightarrow 8$ and $4\beta \rightarrow 6$, which are B-type linkages (**Figure 1e**). The A-type linkage is less common and consists of both $4\beta \rightarrow 8$ and $2\beta \rightarrow O \rightarrow 7$ linkages (**Figure 1f**) (*8*). Degree of polymerization (DP) in reference to procyanidins is the number of monomers linked together through either A- or B-type linkages to form oligomers or polymers.

Cranberries contain high levels of procyanidins, which have been shown to have antioxidant properties due to their reducing ability (*7*). They are also able to bind proteins and metal ions, which may further contribute to their health benefits (*7*). Procyanidins from cranberry have been recognized for anticancer properties and inhibition of bacterial adhesion to the urinary tract and human gastric mucus (*9*, *10*).

†Part of the Berry Health Symposium 2009.

*Corresponding author [telephone (479) 575-2978; fax (479) 575-6936; e-mail lukeh@uark.edu].

 Published on Web 12/18/2009

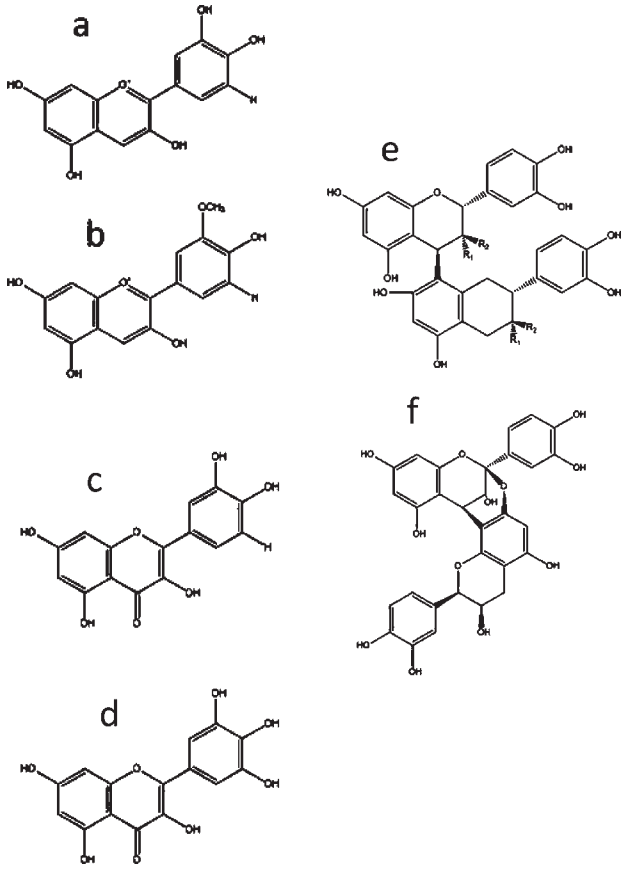

Figure 1. Structures of common polyphenolics found in cranberries: (**a**) cyanidin; (**b**) peonidin; (**c**) quercetin; (**d**) myricetin; (**e**) procyanidin dimer exhibiting a B-type linkage; (**f**) procyanidin dimer exhibiting an A-type linkage.

Although much work has been done to determine polyphenolic compounds and their quantities present in fresh cranberry fruit, there is little qualitative or quantitative information on the compounds that remain in the pomace. Identification and subsequent quantification of the polyphenolics present in cranberry pomace could lead to further studies on extraction of the compounds to be used as nutraceuticals or as natural additives to juices and other beverages, therefore adding the aforementioned health benefits to these products. This paper outlines the proximate composition as well as the identification and quantification of the major polyphenolic compounds present in cranberry pomace.

MATERIALS AND METHODS

Chemicals. HPLC-grade acetone, methanol, acetonitrile, acetic acid, and formic acid were obtained from EMD Biosciences (Madison, WI). Sephadex LH-20 was purchased from Sigma Chemical Co. (St. Louis, MO).

Reference Compounds. Anthocyanin standards of cyanidin-3-glucoside and peonidin-3-glucoside were purchased from Polyphenols Laboratories (Sandnes, Norway). A standard consisting of procyanidin oligomers purified from cocoa was obtained from Masterfoods (Hacketstown, NJ). Quercetin and myricetin standards were purchased from Sigma Aldrich (St. Louis, MO).

Sample. Dried cranberry pomace was obtained from Decas Cranberry Co. (Carver, MA). The pomace was a blend of several cranberry varieties. It was ground to a powder by passage through a 1000 μm sieve screen using a Cyclone sample mill (Udy, Fort Collins, CO) and stored at −70 °C until analysis.

Proximate Analysis. Ground cranberry pomace was analyzed for proximate composition by AOAC methods for moisture (925.10), crude protein (960.52), crude fat (920.39), ash (900.02), and dietary fiber (985.29). Other carbohydrates were determined according to the difference method by subtracting the other components from 100. All analyses were performed in triplicate and values averaged.

Extraction of Polyphenolics. Polyphenolics from cranberry pomace were extracted with acetone/water/acetic acid (70:29.5:0.5 v/v/v) using a Euro Turrax Tissuemizer (Tekmar-Dohrman Corp., Mason, OH). Samples (1 g) were added to 20 mL of solvent, homogenized for 1 min, and filtered through Miracloth (Calbiochem, LaJolla, CA). The residue was collected, and two more extractions were performed. The filtrates were pooled, and the volume of the extract was adjusted to 100 mL with extraction solvent. Extracts were stored at −70 °C until analysis. Extractions were performed in triplicate.

Sephadex LH-20 Isolation of Procyanidins. A cleanup step was conducted according to the methods of Gu et al. (*11*) to eliminate interfering compounds for analysis of procyanidins. Three grams of Sephadex LH-20 was hydrated for 4 h and packed into a 6 × 1.5 cm column. The column was attached to a Sep-Pak vacuum manifold (Waters Corp., Milford, MA) connected to a vacuum pump. Polyphenolic extract (10 mL) was concentrated using a SpeedVac concentrator (ThermoSavant, Holbrook, NY) to remove the acetone from the solvent mixture prior to cleanup. Concentrated extracts were loaded onto the column and eluted with 40 mL of aqueous methanol (30% v/v) to remove sugars, phenolic acids, anthocyanins, and flavonols; this fraction was discarded. The columns were then eluted with 80 mL of aqueous acetone (70% v/v); this fraction, containing procyanidins, was collected.

Reverse-Phase High-Performance Liquid Chromatography (HPLC) Analysis of Anthocyanins. Anthocyanins were analyzed with a Waters Alliance model Delta 600 HPLC (Waters Corp., Milford, MA), equipped with a model 600 pump, a model 717 Plus autosampler, and a model 2996 photodiode array detector. Polyphenolic extract (8 mL) was concentrated, resuspended in 1 mL of 3% formic acid, and filtered through a 0.45 μm filter prior to HPLC analysis. Compounds were separated on a 4.6 × 250 mm Symmetry C_{18} column (Waters Corp.) using the reverse-phase HPLC method described by Cho et al. (*12*). The mobile phase consisted of a linear gradient of 5% formic acid (A) and methanol (B) from 2 to 60% B for 60 min with a flow rate of 1 mL/min. Anthocyanins were detected at 520 nm and were quantified using external calibration curves of a mixture of anthocyanin glucoside standards (Polyphenols Laboratories).

Reverse-Phase Analysis of Flavonols. Flavonols were analyzed with a Waters HPLC system. Polyphenolic extracts (8 mL) were concentrated, resuspended in 1 mL of 50% methanol, and filtered through a 0.45 μm filter prior to HPLC analysis. Compounds were separated using an Aqua 5 μm, 250 × 4.6 mm C_{18} column (Phenomenex, Torrance, CA) using reverse-phase HPLC. A binary gradient consisted of 2% acetic acid (A) and 0.5% acetic acid in water/acetonitrile (1:1 v/v) (B). The linear gradient consisted of 0−50 min, 10−55% B; 50−60 min, 55−100% B; 60−65 min, 100−10% B; 65−70 min, 10% B isocratic. The peaks were monitored at 360 nm, and compounds were quantified as quercetin or myricetin equivalents using external calibration curves of authentic standards.

HPLC Analysis of Procyanidins. Procyanidins were analyzed with a Waters Alliance 2690 HPLC (Waters Corp.) equipped with a Waters model 474 fluorescence detector according to the method of Hammerstone et al. (*13*) with slight modifications. The eluent from the isolation of procyanidins was collected, concentrated, and resuspended in 2 mL of acetone, water, and acetic acid (70:29.5:0.5 v/v/v) and then filtered through a 0.45 μm nylon filter prior to HPLC analysis. Compounds were separated using a 5 μm, 250 × 4.6 mm Luna silica column (Phenomenex). The binary gradient consisted of dichloromethane, methanol, water, and acetic acid [(A) 82:14:2:2; v/v/v/v] and methanol, water, and acetic acid [(B) 96:2:2; v/v/v]. The gradient was as follows: 0−20 min, 0−11.7% B; 20−50 min, 11.7−25.6% B; 50−55 min, 25.6−87.7% B; 55−65 min, 87.7% B isocratic; 65−70 min, 87.7−0% B. This was followed by a 5 min equilibration period. The peaks were monitored by fluorescence detection with excitation at 276 nm and emission at 316 nm, and compounds were quantified on the basis of an external calibration curve of a mixture of standard procyanidins purified from cocoa, which ranged from monomers to decamers (*14*).

High-Performance Liquid Chromatrography−Electrospray Ionization−Mass Spectrometry (HPLC-ESI-MS) of Polyphenols. Individual anthocyanins and flavonols were identified by HPLC-ESI-MS using an HP 1000 series HPLC connected to a Bruker Esquire 2000 quadrupole

Table 1. Proximate Composition of Cranberry Pomace

component	crude content (g/100 g)	component	crude content (g/100 g)
moisture	4.5	insoluble fiber	65.5
protein	2.2	soluble fiber	5.7
fat	12.0	other carbohydrates[a]	8.4
ash	1.1	total polyphenolics	0.6

[a] Carbohydrate content was determined by the difference method.

ion trap mass spectrometer (Bruker Daltronics, Billerica, MA). HPLC separations of the compounds were conducted as previously described. Data were collected in the positive ion electrospray mode for anthocyanin analysis and in the negative ion electrospray mode for flavonols. Conditions of the mass spectrometer were as described by Cho et al. (*12*).

Procyanidins were also identified by HPLC-ESI-MS using an Agilant 1100 series HPLC (Agilent Technologies, Palo Alto, CA) equipped with a UV−vis detector. HPLC separations of the compounds were conducted as previously described. The HPLC was connected to a Bruker Esquire ion trap mass spectrometer (Bruker Daltronics). Conditions of the mass spectrometer were as described by Gu et al. (*8*).

Matrix-Assisted Laser Desorption/Ionization−Time-of-Flight Mass Spectrometry (MALDI-TOF-MS) of Procyanidins. The procyanidin extract resulting from Sephadex LH-20 solid phase extraction was mixed in a 1:1 ratio with 1 M dihydroxybenzoic acid (DHB) in 90% methanol. The sample (2 μL) was spotted onto a ground stainless steal MALDI target using the dry droplet method. Analysis was conducted using a Bruker Reflex III MALDI-TOF-MS. The system was equipped with a 337 nm N_2 laser, and data were obtained in the positive ion reflectron mode.

RESULTS AND DISCUSSION

Proximate Analysis. Cranberry pomace was analyzed for moisture, crude protein, crude fat, and ash. Total carbohydrates were determined by the difference method. The results of proximate analysis are presented in **Table 1**. The pomace was composed mainly of insoluble dietary fiber (65.5%) with smaller portions of fat, soluble fiber, protein, and ash. This was consistent with studies performed on cranberry cell wall material, which found that the majority of the cell wall consisted of cellulose, pectin, and hemicellulose with minor levels of protein, fat, starch, and ash (*15*). The relatively high fat content of cranberry pomace (12.0%) can be attributed to oil from the cranberry seeds and wax present on the skins (*16*).

Anthocyanins. Six anthocyanin glycosides were identified by HPLC-ESI-MS in cranberry pomace. The HPLC chromatogram (**Figure 2**) indicated the presence of six peaks with baseline separation. Mass spectral data used for peak identification and quantification of individual anthocyanins are presented in **Table 2**. The anthocyanins identified were the galactosides, glucosides, and arabinosides of cyanidin and peonidin. Cyanidin 3-arabinoside was the most prominent anthocyanin, followed by peonidin 3-arabinoside, peonidin 3-galacoside, and cyanidin 3-galactoside. Minor amounts of peonidin 3-glucoside and cyanidin 3-glucoside were also observed. This was consistent with anthocyanins identified in fresh cranberries by Wu and Prior (*17*).

Peaks with the same mass spectral fragmentation properties were identified on the basis of HPLC elution order. On the C_{18} column used for anthocyanin separation, compounds eluted in order of decreasing polarity; therefore, the galactoside of cyanidin eluted first, followed by its glucoside and then its arabinoside. This preceded the galactoside, glucoside, and arabinoside of peonidin. The pomace contained a total of 121.4 ± 5.9 mg/100 g of DW anthocyanins. We analyzed fresh cranberries that were either of the variety Howe or Early Black and found that they contained 562 mg/100 g of DW of total anthocyanins. Values in the pomace, however, were comparable to those of a spray-dried cranberry extract, which contained 160 mg/100 g of DW (*18*).

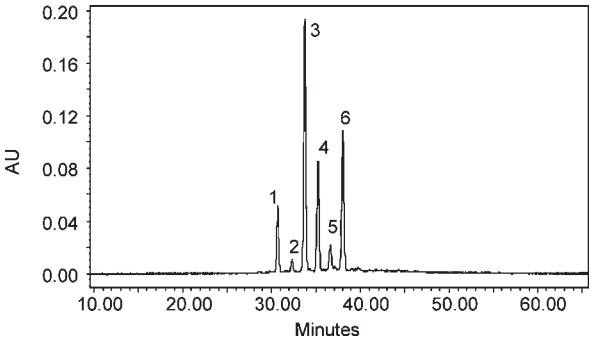

Figure 2. HPLC chromatogram of anthocyanins in cranberry pomace identified by HPLC-ESI-MS and detected at 520 nm. See **Table 2** for peak identification.

Some anthocyanins that are present in fresh cranberries, but do not remain in the cranberry pomace, are expressed in the cranberry juice; however, others may be degraded by heat or enzymes during the mashing step. Anthocyanins are very heat labile (*19*) and may have been degraded due to the heat applied during juice processing. Fresh fruits contain polyphenol oxidase, an enzyme that is responsible for tissue browning by conversion of phenolic substances to quinones, which polymerize to give brown pigments. Upon crushing of fruit during juicing, polyphenol oxidase comes into contact with phenolics, which are released from the vacuoles, causing degradation. Anthocyanins can also be degraded by pectinase enzymes, which are used in the juicing industry to improve juice extraction. Wightman and Wrolstad (*20*) found that enzyme preparations used in cranberry juicing contain glycosidase activity, specifically β-galactosidase activity, capable of destabilizing the anthocyanins by producing aglycones.

Flavonols. Cranberries have one of the highest flavonol contents of all fruits and berries (*21*). The major flavonol in both freeze-dried cranberry powder and fresh fruit is quercetin-3-galactoside, followed by quercetin-3-rhamnopyranoside and quercetin-3-arabinofuranoside in freeze-dried powder and fresh fruit, respectively (*22*, *23*).

Thirteen flavonols were identified in cranberry pomace by HPLC-ESI-MS (**Table 3** and **Figure 3**). Most of the flavonols identified were glycosides of myricetin and quercetin; however, high amounts of myricetin and quercetin as aglycones were identified (55.6 and 146.2 mg/100 g of DW, respectively). Fresh fruit analyzed in our laboratory (Early Black or Howe) contained only 3.71 and 3.65 mg/100 g of DW of myricetin and quercetin, respectively. Because these compounds were not present in high levels in the fresh fruit, they must be a result of processing. As previously mentioned, pectinase enzymes are often used during juice processing to improve juice yield. These pectinase enzyme mixtures have glycosidase activity capable of cleaving the sugar from the flavonol glycoside, leaving the aglycone (*20*). Unlike anthocyanin aglycones, flavonol aglycones are stable enough to be observed by HPLC. Aglycones of myricetin and quercetin were also found in a processed cranberry powder (*22*).

Additionally, two unique flavonols, quercetin coumaroyl galactoside and quercetin benzoyl galactoside, both acylated flavonols, were found in cranberry pomace. These flavonols are not typically found in fresh cranberries and are also likely formed as a result of processing. Quercetin coumaroyl galactoside was found in *Ledum palustre* L. (*24*), whereas quercetin benzoyl galactoside has been reported in a processed cranberry powder (*22*) and only recently for the first time in fresh cranberries (*25*). Mass spectral data indicate two flavonols with fragmentation patterns

Table 2. Peak Identification, Retention Times, Mass Spectral Data, and Quantification of Anthocyanin Glycosides[a] Detected in Cranberry Pomace Using HPLC-ESI-MS

			mass to charge ratios (*m*/*z*)		
peak	retention time (min)	anthocyanin	$[M+H]^+$	fragments	concentration[b] (mg/100 g of DW)
1	30.6	cyanidin-3-galactoside	449	287 [M − galactose]	13.2 ± 0.2
2	32.2	cyanidin-3-glucoside	449	287 [M − glucose]	4.5 ± 0.2
3	33.6	cyanidin-3-arabinoside	419	287 [M − arabinose]	49.6 ± 6.8
4	35.2	peonidin-3-galactoside	463	301 [M − galactose]	20.1 ± 0.5
5	36.7	peonidin-3-glucoside	463	301 [M − glucose]	7.4 ± 0.3
6	38.1	peonidin-3-arabinoside	433	301 [M − arabinose]	26.6 ± 0.5
		total			121.4 ± 5.9

[a] Data expressed as equivalents of cyanidin or peonidin glucosides. [b] Mean ± standard deviation (n = 3).

Table 3. Peak Identification, Retention Times, Mass Spectral Data, and Quantification of Flavonols[a] Detected in Cranberry Pomace Using HPLC-ESI-MS

			mass to charge ratios (*m*/*z*)		
peak	retention time (min)	flavonol	$[M-H]^-$	fragments	concentration[b] (mg/100 g of DW)
1	36.4	myricetin 3-xyloside	449	316 [M − xylose]	1.5 ± 0.3
2	37.3	myricetin 3-arabinoside	449	316 [M − arabinose]	1.8 ± 0.1
3	38.3	quercetin 3-galactoside	463	300 [M − galactose]	12.8 ± 3.6
4	41.0	quercetin 3-xyloside	433	300 [M − xylose]	5.5 ± 0.3
5	42.0	quercetin 3-arabinopyranoside	433	300 [M − arabinose]	15.2 ± 3.6
6	43.4	quercetin 3-arabinofuranoside	433	300 [M − arabinose]	16.7 ± 3.5
7	44.2	quercetin 3-rhamnoside	447	300 [M − rhamnose]	18.5 ± 3.4
8	48.0	myricetin	317	(myricetin) 315 [M − xylose]	55.6 ± 2.6
9	49.0	methoxyquercetin 3-xyloside	447	300 [M − xylose + methoxy]	11.4 ± 3.7
10	55.1	quercetin 3-coumaroyl galactoside	609	463 [M − galactose], 300 [M − coumaric acid + galactose]	2.3 ± 0.3
11	55.7	unidentified	583	316	12.1 ± 3.5
12	58.1	quercetin	300	(quercetin)	146.2 ± 22.7
13	58.7	quercetin 3-benzoyl galactoside	567	300	27.5 ± 3.4
		total			358.4 ± 16.3

[a] Flavonols are expressed as quercetin or myricetin equivalents. [b] Mean ± standard deviation (n = 3).

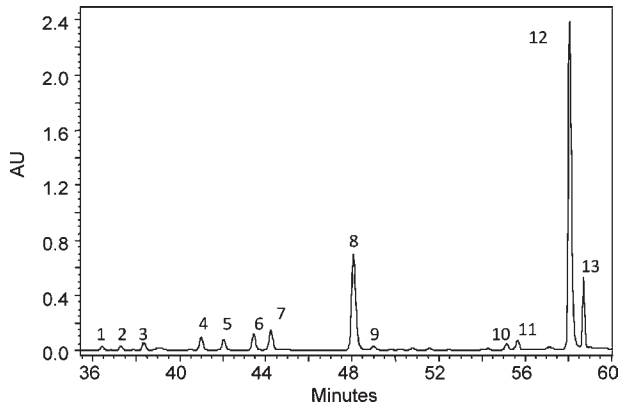

Figure 3. HPLC chromatogram of flavonols in cranberry pomace identified by HPLC-ESI-MS and detected at 360 nm. See **Table 3** for peak identification.

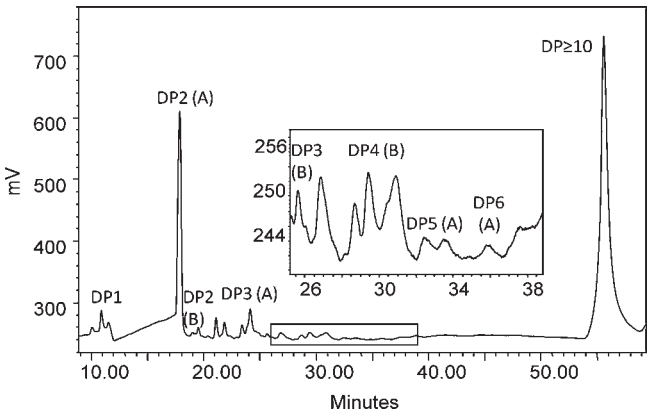

Figure 4. HPLC chromatogram of procyanidins in cranberry pomace identified by HPLC-ESI-MS and detected by fluorescence with ex 276 nm and em 316 nm. See **Table 4** for peak identification.

corresponding to quercetin 3-arabinoside in cranberry pomace, indicating
different conformations of the same flavonol. Vvedenskaya et al. (*22*) identified quercetin 3-arabinoside in both the pyranose and furanose forms in cranberry powder using nuclear magnetic resonance.

The pomace contained 358.4 ± 16.3 mg/100 g of DW total flavonols. To compare this to the fresh fruit, fresh cranberries (Early Black or Howe) were analyzed in our laboratory for flavonols and found to contain 651.5 mg/100 g of DW. This suggests that appreciable levels of flavonols from fresh cranberries were not expressed in the juice; that is, they were retained in the pomace, and are stable during processing.

Procyanidins. Cranberry pomace was analyzed for the type and size of procyanidins present. The HPLC profile with fluorescence detection of procyanidins in cranberry pomace is presented in **Figure 4**, and data on the identification and quantification of procyanidins can be found in **Table 4**. The HPLC chromatogram indicates the presence of a small amount of momomeric epicatechin and catechin (DP1), which was confirmed using

Table 4. Peak Identification, Mass Spectral Data, and Quantification of Procyanidins Detected in Cranberry Pomace by HPLC-ESI-MS

peak	procyanidin	mass to charge ratio (*m*/*z*)	concentration[a] (mg/100 g of DW)
DP1	monomers (catechin/epicatechin)	289	5.8 ± 0.4
DP2 (A)	A-type dimer[b]	575	82.6 ± 2.3
DP2 (B)	B-type dimer	577	4.4 ± 0.7
DP3 (A)	A-type trimer[c]	863	30.8 ± 2.5
DP3 (B)	B-type trimer	865	1.6 ± 0.4
DP4 (B)	B-type tetramer	1152	22.9 ± 3.6
DP5 (A)	A-type pentamer[d]	1439	7.1 ± 1.2
DP6 (A)	A-type hexamer[e]	1721	12.1 ± 1.3
	total oligomers		167.3 ± 5.9

[a] Means ± standard deviation ($n = 3$). [b] Data expressed as B-type dimer equivalents. [c] Data expressed as B-type trimer equivalents. [d] Data expressed as B-type pentamer equivalents. [e] Data expressed as B-type hexamer equivalents.

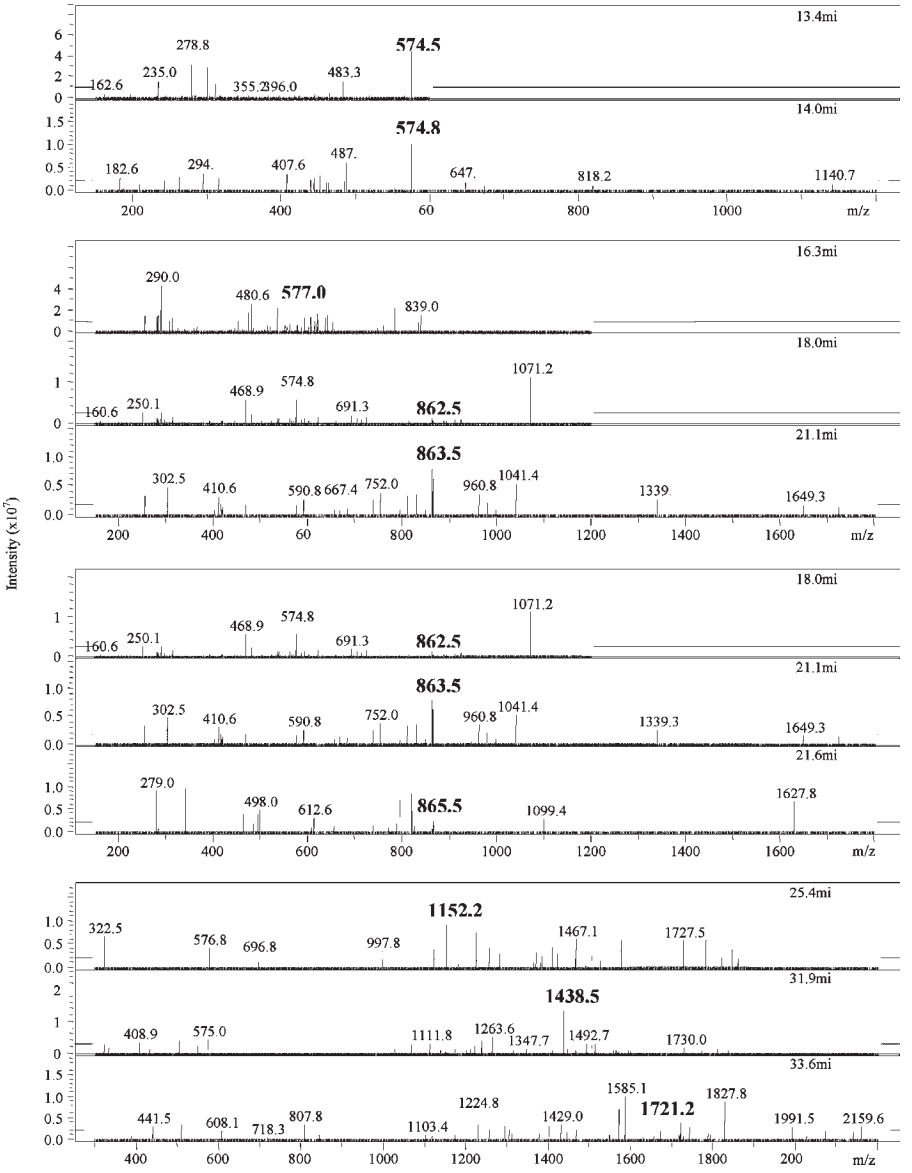

Figure 5. HPLC-ESI-MS spectra of procyanidins in cranberry pomace (DP2−DP6) obtained in the negative ion mode. A-type dimers = *m*/*z* 575; B-type dimers = *m*/*z* 577; A-type trimers = *m*/*z* 863; B-type trimers = *m*/*z* 865; B-type tetramers = *m*/*z* 1152; A-type pentamers = *m*/*z* 1439; A-type hexamers = *m*/*z* 1721.

external standards. The mass spectral data (**Figure 5**) indicate the presence of both A- and B-type linkages in the procyanidins. An A-type dimer was the primary procyanidin, with minor levels of A-type trimers, tetramers, and higher oligomers. These findings were consistent with those of Gu et al. (*8*), who used LC-MS/MS on an extract from whole cranberries to identify several procyanidin oligomers in cranberry containing A-type linkages and the location of the linkage. The researchers also performed thiolytic degradation of procyanidins in the cranberries to determine their DP. Excluding the monomeric flavan-3-ols, the average DP was 8.5 and, including the monomers, the DP was 8.3.

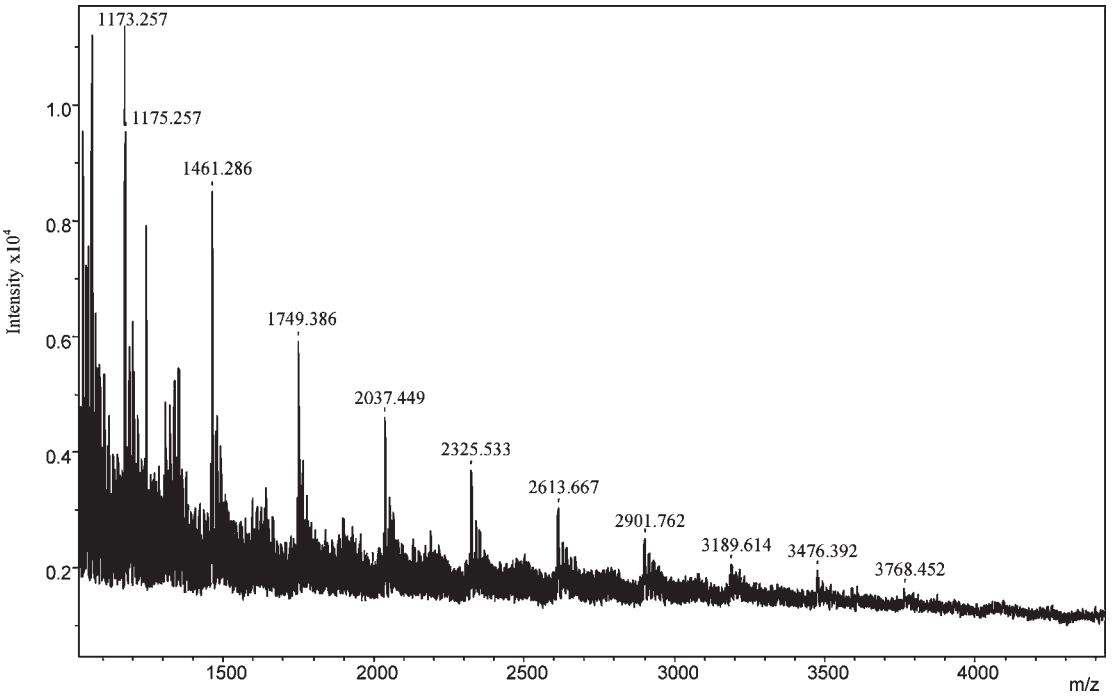

Figure 6. MALDI-TOF-MS spectrum of procyanidins in cranberry pomace.

MALDI-TOF-MS was also used to identify procyanidins in cranberry pomace (**Figure 6**). This allowed for identification of high molecular weight procyanidins present in the pomace (DP4−DP13), which could not be identified by ESI-MS due to their size. Each of the peaks represents sodium adducts of procyanidin oligomers containing various amounts of A-type linkages. The compound with a molecular weight of 1173.3 represents a sodium adduct of a DP4 procyanidin containing two A-type linkages, whereas the compound with a molecular weight of 1175.3 represents a sodium adduct of a DP4 procyanidin containing only one A-type linkage. A compound containing an A-type linkage is 2 mass units smaller than the same DP procyanidin containing only B-type linkages, and each additional A-type linkage would result in a loss of 2 more mass units. MALDI-TOF-MS also revealed the presence of DP5−DP12 procyanidins containing two A-type linkages. Additionally, the compound with a molecular weight of 3768.5 represents the sodium adduct of a DP13 procyanidin containing no A-type linkages. Reed et al. (*26*) also identified procyanidins in a cranberry presscake that were fractionated using Sephadex LH-20. They identified procyanidins containing DP5−DP23 in the aqueous acetone fraction, whereas we identified procyanidins with DP4−DP13 in the aqueous acetone fraction. They also identified procyanidins in the aqueous methanol fraction; however, we discarded this fraction. They identified compounds that were 16 mass units greater than predicted procyanidins, indicating the possibility of oligomers containing one epigallocatechin unit. However, our MALDI-TOF MS data did not indicate the presence of any such compounds, but it is possible that these compounds were discarded in the aqueous methanol fraction. Our MALDI-TOF spectrum more closely resembles the one presented by Neto et al. (*27*).

The pomace contained 167.3 mg/100 g of DP1−DP6 procyanidins on a dry pomace weight basis. These data may not be representative of all of the procyanidins present in cranberry pomace, as appreciable levels may be unextractable and the polymers (DP > 10) were not quantified due to the lack of a commercial standard. Recent studies have indicated that many of the procyanidins are highly bound to plant cell wall material and, therefore, unextractable (*28*, *29*). Because cranberry pomace is composed primarily of dietary fiber (cell wall material), it is possible that many of the polyphenolic compounds, including procyanidins may be enclosed within this portion of the pomace. Therefore, many of the polyphenolics may be destined for the gastrointestinal tract, where they would be subjected to microbial metabolism. Some studies have attempted to measure the amount of unextractable procyanidins in plant products using acid-catalyzed degradation by butanol−HCl or thiolysis and have found that significant amounts of procyanidins in many plant materials are indeed unextractable under normal extraction conditions (*30*−*32*). Therefore, new extraction procedures need to be developed that facilitate the release of procyanidins from cell wall residue.

ACKNOWLEDGMENT

We thank Decas Cranberry Co., Inc., for providing the cranberry pomace used in this study. We also thank Rohana Liyanage and the University of Arkansas Statewide Mass Spectrometry Facility for assistance and use of the MALDI-TOF-MS instrument.

LITERATURE CITED

(1) Vattem, D. A.; Shetty, K. Solid-state production of phenolic antioxidants from cranberry pomace by *Rhizopus oligosporus*. *Food Biotechnol*. **2002**, *16*, 189–210.

(2) Kahlon, T. S.; Smith, G. E. In vitro binding of bile acids by blueberries (*Vaccinium* spp.), plums (*Prunus* spp.), prunes (*Prunus* spp.), strawberries (*Fragaria* × *ananassa*), cherries (*Malpighia punicifolia*), cranberries (*Vaccinium macrocarpon*) and apples (*Malus sylvestris*). *Food Chem*. **2007**, *100*, 1182–1187.

(3) Chu, Y.; Liu, R. H. Cranberries inhibit LDL oxidation and induce LDL receptor expression in hepatocytes. *Life Sci*. **2005**, *77*, 1892–1901.

(4) Seeram, N. P.; Adams, L. S.; Hardy, M. L.; Heber, D. Total cranberry extract vs its phytochemical consituents: antiproliferative and synergistic effects against human tumor cell lines. *J. Agric. Food Chem*. **2004**, *52*, 2512–2517.

(5) Manach, C.; Scalbert, A.; Morand, C.; Remesy, C.; Jimenez, L. Polyphenols: food sources and bioavailability. *Am. J. Clin. Nutr.* **2004**, *79*, 727–747.
(6) Skrede, G.; Wrolstad, R. E. Flavonoids from berries and grapes, In *Functional Foods: Biochemical and Processing Aspects*, 1st ed.; Shi, J., Mazza, G., Maguer, M. L., Eds.; CRC Press: Boca Raton, FL, 2002; Vol. 2, pp 71−133.
(7) Santos-Buelga, C.; Scalbert, A. Proanthocyanidins and tannin-like compounds − nature, occurrence, dietary intake and effects on nutrition and health. *J. Sci. Food Agric.* **2000**, *80*, 1094–1117.
(8) Gu, L.; Kelm, M. A.; Hammerstone, J. F.; Beecher, G.; Holden, J.; Haytowitz, D.; Prior, R. L. Screening of foods containing proanthocyanidins and their structural characterization using LC-MS/MS and thiolytic degradation. *J. Agric. Food Chem.* **2003**, *51*, 7513–7521.
(9) Foo, L. Y.; Lu, Y.; Howell, A. B.; Vorsa, N. A-type proanthocyanidin trimers from cranberry that inhibit adherence of uropathogenic p-fimbriated *Escherichia coli*. *J. Nat. Prod.* **2000**, *63*, 1225–1228.
(10) Kandil, F. E.; Smith, M. A.; Rogers, R. B.; Pepin, M. F.; Song, L. L.; Pezzuto, J. M.; Seigler, D. S. Composition of a chemopreventive proanthocyanidin-rich fraction from cranberry fruits responsible for the inhibition of 12-*O*-tetradecanoyl phorbol-13-acetate (TPA)-induced ornithine decarboxylase (ODC) activity. *J. Agric. Food Chem.* **2002**, *50*, 1063–1069.
(11) Gu, L.; Kelm, M.; Hammerstone, J. F.; Beecher, G.; Cunningham, D.; Vannozzi, S.; Prior, R. L. Fractionation of polymeric procyanidins from lowbush blueberry and quantification of procyanidins in selected foods with an optimized normal-phase HPLC-MS fluorescent detection method. *J. Agric. Food Chem.* **2002**, *50*, 4852–4860.
(12) 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.
(13) Hammerstone, J. F.; Lazarus, S. A.; Mitchell, A. E.; Rucker, R.; Schmitz, H. H. Identification of procyanidins in cocoa (*Theobroma cacao*) and chocolate using high-performance liquid chromatography/mass spectrometry. *J. Agric. Food Chem.* **1999**, *47*, 490–496.
(14) Kelm, M. A.; Johnson, J. C.; Robbins, R. J.; Hammerstone, J. F.; Schmitz, H. H. High-performance liquid chromatography separation and purification of cacao (*Theobroma cacao* L.) procyanidins according to degree of polymerization using a diol stationary phase. *J. Agric. Food Chem.* **2006**, *54*, 1571–1576.
(15) Holmes, A. B.; Rha, C. Structure and chemical composition of cranberry cell wall material. *J. Food Sci.* **1978**, *43*, 112–120.
(16) Fellers, C. R. Nutritive value of cranberries. *Am. J. Public Health Nations Health* **1933**, *23*, 13–18.
(17) 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.
(18) 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.
(19) Attoe, E. L.; Von Elbe, J. H. Photochemical degradation of betanine and selected anthocyanins. *J. Food Sci.* **1981**, *46*, 1934–1937.
(20) Wightman, J. D.; Wrolstad, R. E. Anthocyanin analysis as a measure of glycosidase activity in enzymes for juice processing. *J. Food Sci.* **1995**, *60*, 862–867.
(21) Häkkinen, S.; Heinonen, M.; Kärenlampi, S.; Mykkänen, H.; Ruuskanen, J.; Törrönen, R. Screening of selected flavonoids and phenolic acids in 19 berries. *Food Res. Int.* **1999**, *32*, 345–353.
(22) Vvedenskaya, I. O.; Rosen, R. T.; Guido, J. E.; Russell, D. J.; Mills, K. A.; Vorsa, N. Characterization of flavonols in cranberry (*Vaccinium macrocarpon*) powder. *J. Agric. Food Chem.* **2004**, *52*, 188–195.
(23) Yan, X.; Murphy, B. T.; Hammond, G. B.; Vinson, J. A.; Neto, C. C. Antioxidant activities and antitumor screening of extracts from cranberry fruit (*Vaccinium macrocarpon*). *J. Agric. Food Chem.* **2002**, *50*, 5844–5849.
(24) Jin, C.; Strembiski, W.; Kulchytska, Y.; Micetich, R. G.; Danshtalab, M. Flavonoid glycosides from *Ledum palustre* L. subsp. Decumbens (AIT) Hulton. *Daru, J. Fac. Pharm., Tehran Univ. Med. Sci.* **1999**, *7*, 5–8.
(25) Singh, A. P.; Wilson, T.; Kalk, A. J.; Cheong, J.; Vorsa, N. Isolation of specific cranberry flavonoids for biological activity assessment. *Food Chem.* **2009**, *116*, 963–968.
(26) Reed, J. D.; Krueger, C. G.; Vestline, M. M. MALDI-TOF mass spectrometry of oligomeric food polyphenols. *Phytochemistry* **2005**, *66*, 2248–2263.
(27) Neto, C. C.; Krueger, C. G.; Lamoureaux, T. L.; Kondo, M.; Vaisberg, A. J.; Hurta, R. A. R.; Curtis, S.; Matchett, M. D.; Yeung, H.; Sweeney, M. I.; Reed, J. D. MALDI-TOF-MS characterization of proanthocyanidins from cranberry fruit (*Vaccinium macrocarpon*) that inhibit tumor cell growth and matrix metalloproteinase expression *in vitro*. *J. Sci. Food Agric.* **2006**, *86*, 18–25.
(28) Le Bourvellec, C.; Guyot, S.; Renard, C. M. G. C. Non-covalent interaction between procyanidins and apple cell wall material: part I. Effect of some environmental parameters. *Biochim. Biophys. Acta* **2004**, *1672*, 192–202.
(29) Le Bourvellec, C.; Bouchet, B.; Renard, C. M. G. C. Non-covalent interaction between procyanidins and apple cell wall material. Part III: Study on model polysaccharides. *Biochim. Biophys. Acta* **2005**, *1725*, 10–18.
(30) Hellström, J. K.; Mattila, P. H. HPLC determination of extractable and unextractable proanthocyanidins in plant materials. *J. Agric. Food Chem.* **2008**, *56*, 7617–7624.
(31) Hellström, J. K.; Törrönen, A. R.; Matilla, P. H. Proanthocyanidins in common food products of plant origin. *J. Agric. Food Chem.* **2009**, *57*, 7899–7906.
(32) Ossipova, S.; Ossipov, V.; Haukioja, E.; Loponen, J.; Pihlaja, K. Proanthocyanidins of mountain birch leaves: quantification and properties. *Phytochem. Anal.* **2001**, *12*, 128–133.

Received for review August 12, 2009. Revised manuscript received October 28, 2009. Accepted November 19, 2009.