

Chemical Composition of Caneberry (*Rubus* spp.) Seeds and Oils and Their Antioxidant Potential

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Caneberries (*Rubus* spp. L.) are grown primarily throughout the Pacific Northwestern United States and Canada. Processing of caneberry fruit typically removes the seed, and the development of a value-added use of seeds could expand the market for caneberries and the profit margins for growers. An initial step toward the use of the seeds is a characterization of seed and oil. Our investigation has described compositional characteristics for seeds of five commonly grown caneberry species: red raspberry, black raspberry, boysenberry, Marion blackberry, and evergreen blackberry. Seeds from all five species had 6–7% protein and 11–18% oil. The oils contained 53–63% linoleic acid, 15–31% linolenic acid, and 3–8% saturated fatty acids. The two smaller seeded raspberry species had higher percentages of oil, the lowest amounts of saturated fatty acid, and the highest amounts of linolenic acid. Antioxidant capacities were detected both for whole seeds and for cold-pressed oils but did not correlate to total phenolics or tocopherols. Ellagitannins and free ellagic acid were the main phenolics detected in all five caneberry species and were approximately 3-fold more abundant in the blackberries and the boysenberry than in the raspberries.

KEYWORDS: Tocopherols; ellagic acid; ellagitannin; α -linolenic acid; linoleic acid; saturated fatty acid; antioxidant capacity; oxygen radical absorbance capacity (ORAC); raspberry; blackberry; boysenberry; Marion blackberry; *Rubus*

INTRODUCTION

Caneberries are *Rubus* species that grow on a leafy cane and produce multiple small fruits such as raspberries and blackberries. Grown in temperate regions, caneberries are prevalent throughout the Northwestern United States. In 2003, over 8000 Ha of caneberries were grown in the United States, with a total production of 62 143 Mt and a market value of approximately \$68M (1). For raspberries, 74 907 Ha was grown worldwide in 2002, at an approximate yield of 411 851 Mt (2). The processing of caneberry fruit for juices and puree typically removes the seed as a byproduct. The development of a value-added use of seeds could expand the market for caneberry products and increase grower profit margins. It is estimated that over 180 000

kg of berry seeds are available in Oregon and Washington from seedless processing (3).

The majority of information regarding caneberry seed has concerned red raspberry (*Rubus idaeus* L.). Red raspberry seed was reported to contain 12.2% protein and 11–23% oil (4–6). The composition of red raspberry seed oil was 54.5% linoleic acid (18:2), 29.1% α -linolenic acid (18:3), 12% oleic acid (18:1), and 4% saturated fatty acids (4, 6). The percentage of α -linolenic acid is similar to hemp, black currant, and cranberry oils (7, 8) and may have utility based on potential health benefits (9, 10). Considerable amounts of tocopherols were found in the red raspberry oil, mainly of γ -tocopherol (4). Tocopherols are common lipophilic antioxidants abundant in some oils and nuts (11), but their presence in red raspberry seed could provide vitamin E activity and antioxidant potential as well (12). Ellagic acid was reported to be more abundant in red raspberry and blackberry (*Rubus* spp.) than in other fruits and nuts (13). Occurring primarily in the seed (11), ellagic acid has shown chemopreventative activity in animal models (14, 15). These characteristics of red raspberry seed suggest possible roles in

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human nutritional products, such that an investigation of seed properties should be extended to other caneberries.

This investigation was carried out to analyze compositional characteristics of red raspberry (*R. idaeus* L.), black raspberry (*Rubus occidentalis* L.), boysenberry (*Rubus ursinus* × *idaeus*), Marion blackberry (*Rubus ursinus*), and evergreen blackberry (*Rubus laciniatus* Willd) seeds. These species represent the most commonly grown caneberries in Oregon and provide a sample of the *Rubus* diversity. Oil and protein yields, fatty acid compositions, antioxidant capacities, tocopherol content and composition, and ellagic acid amounts are reported for each species on a whole seed basis.

MATERIALS AND METHODS

Sample Preparations. Seeds from five caneberry species were received as frozen pulp from industrial sources. Red raspberry pulp was received as a combination of *Willamette* and *Meeker* varieties. The red raspberry and *Marion* variety blackberry seeds were received from Scenic Fruit Co. (Gresham, OR). Black raspberry pulp was comprised of the *Munger* variety and was received from Decker Farms (Hillsboro, OR). The boysenberry and evergreen blackberry are the variety names of the respective species, and the pulp of these varieties was received from RainSweet, Inc. (Salem, OR). The pulp was oven-dried at 50 °C for 18 h, threshed with a standard seed thresher, and cleaned via forced air. Cold-pressed oil was extracted from the seeds using a screw-press (Botanic Oil Innovation, Spooner, WI). Extrusion of the oil took place at the press head under pressures up to 9652 kPa, and oil was pressed from the seed material in temperature ranges of 27–38 °C. The oil concentration was determined using butt extraction on ground seeds on a dry weight basis by American Oil Chemists' Society (AOCS) method Ba3-38, and moisture percent was determined using AOCS method Ba2a-38. The protein concentration was determined by the Association of Analytical Chemists (AOAC) method 990.03 (1995) with a Leco CHN analyzer (Leco Corp., St. Joseph, MI). The amino acid profile was determined by AOAC method 982.30 (1995) using a Beckman 6300 amino acid cationic analyzer (Beckman Coulter Inc., Fullerton, CA).

Fatty Acid Composition. The fatty acid composition was analyzed by *trans*-esterification of 10 mg of oil with 0.5 M sodium methoxide (0.5 mL) directly from the seed at 50 °C for 30 min in a sealed crimp cap vial. The solution was quenched with 0.5 mL of 0.5 M HCl, after first diluting in 2 mL of hexane. The hexane layer was withdrawn, placed in a 2 mL crimp top vial, and injected directly onto the gas chromatograph (GC). GC analyses were performed on a BPX-70 polar 0.25 mm × 30 m column (J&W Scientific Co., Folsom, CA). The temperature ramp was from 150 to 250 °C at 5 °C/min with the injector and detector set at 250 °C. Saturated C8 to C30 fatty acid methyl esters provided standards for calculating equivalent chain length values used to make fatty acid methyl ester assignments.

Phenolic Analyses. For the estimation of total phenolics, ground berry seeds were extracted in methanol for 1 h at room temperature and then centrifuged at 3700 rcf. Total phenolics were estimated from the supernatant according to the Folin–Ciocalteu procedure (16) on a COBRA FARA II centrifugal analyzer (Roche Diagnostic System Inc., Branchburg, NJ), with gallic acid as an external standard. The high-performance liquid chromatography (HPLC) analyses of phenolic extracts were conducted on ground berry seeds extracted in 70% acetone, vortexed, and centrifuged at 15300 rcf for 5 min. Two volumes of chloroform were added to the supernatant, and 500 µL of the top aqueous layer was removed. The samples were diluted 10-fold, and 20 µL was injected into a Synergi Hydro-RP column using a Varian 5020 Liquid Chromatographic system and Agilent 1100 autosampler (Agilent Technologies, Palo Alto, CA). Samples were resolved in 90% formic acid:10% methanol for 50 min, 65% formic acid:35% methanol for 5 min, 35% formic acid:65% methanol for 6 min, and 90% formic acid:10% methanol for 4 min. Readings were taken at 260, 280, 320, 365, and 520 nm wavelengths with a Hewlett-Packard 1040A Photodiode Array Detector (Hewlett-Packard, Palo Alto, CA). Gallic acid, chlorogenic acid, catechin, epicatechin, and ellagic acid were used as

standards. For alkaline hydrolysis, samples were saponified in 9% potassium hydroxide for 10 min at room temperature in the dark. The solutions were neutralized with 9 mL of 2 N HCl, and the hydrolysates were loaded onto Sep-Pak C₁₈ cartridges (Millipore, Billerica, MA). Each cartridge was washed twice with acidic water and then eluted with 9 mL of methanol. The samples were then evaporated and dissolved in 1 mL of water. The solutions were then filtered through a 45 µm Millipore filter, and 20 µL was injected into the HPLC column.

Ellagic Acid Analyses. Five grams of the ground seed was extracted by methanol at 100 °C for 24 h (containing free ellagic acid), and the extract was then evaporated to dryness and hydrolyzed in 2 N trifluoroacetic acid in methanol at 100 °C for 2 h (containing total ellagic acid). The quantitation of ellagic acid was performed using a Phenomenex (Torrance, CA) 5 µm Phenyl Hexyl column (250 mm × 4.6 mm) at the wavelength of 280 nm. The binary mobile phase consisted of (A) water, acetonitrile, and acetic acid (89:9:2, v/v) and (B) water and acetonitrile (20:80,v/v). The gradient method started at 1 mL/min from 100% A for 25 min and then was linearly changed to 100% B over 15 min.

Oxygen Radical Absorbance Capacity (ORAC). ORAC assays were conducted by Brunswick Laboratories (Wareham, MA) on whole seed and expeller-pressed oil. The whole seed hydrophilic ORAC analyses were based on a procedure of Ou et al. (17). Briefly, 0.5 g of ground seed was added to 20 mL of acetone/water (50:50 v/v), and the mixture was shaken at room temperature for 1 h. The samples were centrifuged at 3700 rcf, and the aqueous extract was analyzed on a Synergy HI microplate fluorescence reader coupled with a Precision 2000 liquid handling system (Bio-Tek Instruments, Inc., Winooki, VT) (18). For lipophilic ORAC assays, expeller-pressed oil was dissolved in acetone and 7% randomly methylated β-cyclodextrin. The lipophilic ORAC was based on a procedure of Huang et al. (19). The ORAC values represent the area under a time curve of protein degradation using the fluorescein probe (17,19), and 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (trolox) was used as an external standard. Thus, values are presented as µM trolox equivalents (TE)/g seed.

Tocopherols. Tocopherol quantification was based on a method by Podda et al. (20). For each sample, 250 mg of powdered whole seed was saponified at 70 °C in 2.5 mL of 1% ascorbic acid, 5 mL of 95% ethanol, and 1.5 mL of saturated KOH for 30 min. Samples were cooled to room temperature; whereupon, 2.5 mL of 1% (w/v) ascorbic acid and 50 µL of butylated hydroxy toluene (1 mg/mL in ethanol) were added. The samples were then extracted with 5 mL of hexane, vacuum-dried, and resuspended in a 1:1 volume methanol:ethanol. From these samples, 50 µL was injected through a Develosil RP-AQUEOUS column (Phenomenex) in a 99:1 methanol:water ratio, using a Waters 2690 separation module. The peaks were detected at 292 nm with a photodiode array detector (Waters Corp., Milford, MA). External standard curves were made with pure α-, β-, γ-, and δ-tocopherols (Matreya Inc., Pleasant Gap, PA).

RESULTS

Seeds from red and black raspberries had 1000-seed weights of approximately 1.5 g, while the other three caneberries were 2.4 (Marion blackberry) and 3.6 (boysenberry and evergreen blackberry) g. The oil concentration and seed weight were negatively correlated ($r = -0.87$; $P = 0.06$). The protein content of the caneberry seeds ranged from 6 to 7%. The amino acid compositions were similar across all five species (Table 1). Methionine and cysteine levels were 4% of the total protein; lysine was also approximately 4% of the total. Total seed oil percentages ranged from 11.4% in boysenberry seed to 18.7% in red raspberry seed. Black raspberry, Marion blackberry, and evergreen blackberry seeds contained 15, 14, and 13.3% oil, respectively.

The relative abundance of fatty acids was similar among all five species (Table 2). Linoleic acid (18:2) comprised 52–63% of the total fatty acids, and α-linolenic acid (18:3) comprised 15–31% of the total. The remaining fatty acids were mainly

Table 1. Caneberry Seed Amino Acid Composition

amino acid	mg/100 g				
	red raspberry	black raspberry	Marion blackberry	boysenberry	evergreen blackberry
glutamic acid	1.40	1.60	1.56	1.33	1.48
aspartic acid	0.68	0.76	0.69	0.64	0.72
arginine	0.54	0.58	0.58	0.54	0.59
leucine	0.47	0.49	0.46	0.44	0.49
glycine	0.40	0.40	0.44	0.40	0.48
alanine	0.33	0.34	0.30	0.36	0.31
valine	0.33	0.34	0.32	0.31	0.35
isoleucine	0.32	0.34	0.32	0.30	0.35
lysine	0.3	0.32	0.29	0.29	0.30
phenylalanine	0.28	0.29	0.27	0.26	0.30
proline	0.27	0.31	0.27	0.26	0.32
serine	0.27	0.27	0.25	0.23	0.25
threonine	0.23	0.24	0.22	0.22	0.23
histidine	0.18	0.19	0.20	0.19	0.20
cysteine	0.17	0.19	0.18	0.14	0.16
tyrosine	0.14	0.15	0.14	0.13	0.15
methionine	0.14	0.14	0.14	0.12	0.13
hydroxyproline	0.04	0.04	0.03	0.04	0.06
tryptophan	0.04	0.04	0.04	0.04	0.04
taurine	0.01	0.06	0.06	0.05	0.05
ornithine	0.01	0.01	0.01	0.01	0.01

oleic (18:1), palmitic (16:0), and stearic (18:0) acids. Other, less common fatty acids were also in the caneberry oils (**Table 2**). A trace amount of C20 fatty acid was present in all five berry seeds. The red and black raspberry seeds had a small amount of 6,9,12-octadecatrienoic acid (γ -linolenic acid). A small amount of δ -6 unsaturation was found in C18:1 acids of black raspberry as petroselinic acid (18:1 Δ 6).

Saturated fatty acids comprised approximately 3% of the total fatty acids in the two raspberries and 5–8% in evergreen

blackberries, boysenberries, and Marion blackberries. Thus, the two raspberries had lower percentages of oleic acid and the highest percentages of α -linolenic acid. Conversely, the blackberries, evergreen and Marion, had higher amounts of saturated fat, lower percentages of α -linolenic acid, and higher percentages of oleic acid. Boysenberry is a *Rubus* hybrid containing red raspberry and blackberry parentage. The fatty acid composition of boysenberry was intermediate between raspberries and blackberries (**Table 2**).

The five caneberries contained total tocopherols in the range of 10–33 mg/100 g seed (**Table 3**). γ -Tocopherol was the most abundant form in all five caneberry seeds, but relatively large amounts of α -tocopherol were also present in raspberries. The levels of δ -tocopherol and β -tocopherol were absent or detected in trace amounts. Assuming that all of the tocopherols remain with the oil during the extraction process, the red raspberry, black raspberry, boysenberry, and evergreen blackberry seed oils contained 153–175 mg/g oil. Marion blackberry seeds had the least tocopherols (73 mg/g oil). ORAC tests were performed on expeller-pressed oils (**Table 3**). The Marion blackberry ORAC value of 119.7 was significantly higher than the other caneberry oils and over twice as large as raspberry ORAC values. The correlation between tocopherols and oil ORAC was nonsignificant ($P = 0.15$).

The antioxidant capacity of whole seeds was assessed with ORAC tests. We found the ORAC values in the range of 146–540 μ M TE/g seed (**Table 4**); red raspberry seeds in particular had over twice the ORAC value of the other caneberries at 539.7 μ M trolox/g seed. As phenolics have been shown to correlate with ORAC values (22), we estimated the total phenolics of whole seeds (**Table 4**). Red raspberry seeds had the highest amount of total phenolics, but the relationship between ORAC and total phenolics was not significant ($R^2 = 0.58$; $P = 0.13$).

Table 2. Fatty Acid Composition in Caneberry Seeds as a Percent of Total

fatty acid	% fatty acid				
	red raspberry	black raspberry	boysenberry	Marion blackberry	evergreen blackberry
16:0 (palmitic acid)	2.4	1.9	3.5	3.4	4.5
16:1	0.1				
16:2	0.1				
18:0 (stearic acid)	0.9	0.8	1.5	2.1	3.3
18:1 Δ 6		0.1			
18:1 Δ 9 (oleic acid)	11.0	10.4	11.6	15.1	17.3
18:1 Δ 11	0.7	0.6	0.7	0.7	0.7
18:2 (linoleic acid)	54.2	53.5	59.1	62.7	53.1
18:3 Δ 6,9,12	0.2	0.2			
18:3 Δ 9,12,15	29.7	31.2	22.1	15.2	19.9
(α -linolenic acid)					
20:0	0.4	0.4	0.7	0.5	0.8
20:1	0.2	0.2	0.5	0.3	0.3
20:2		0.1	0.1		
22:0	0.1	0.1	0.2		

Table 3. Means and Standard Errors of Tocopherol Composition in Whole Seeds and ORAC on Cold-Pressed Seed Oils

source	α -tocopherol (mg/100 g seed)	γ -tocopherol (mg/100 g seed)	total ^a tocopherol (mg/100 g seed)	ORAC _{ipoFL} (μ M TE ^b /g oil)
red raspberry	12.6 \pm 0.014	19.4 \pm 0.017	33.0 \pm 0.027	53.67 \pm 4.17
black raspberry	8.3 \pm 0.024	16.3 \pm 0.041	26.2 \pm 0.068	52.33 \pm 2.13
boysenberry	2.0 \pm 0.003	15.4 \pm 0.080	17.5 \pm 0.084	56.67 \pm 4.74
Marion blackberry	1.6 \pm 0.006	8.5 \pm 0.024	10.2 \pm 0.030	119.67 \pm 3.85
evergreen blackberry	2.0 \pm 0.000	20.7 \pm 0.025	23.3 \pm 0.026	88.33 \pm 2.33

^a Trace amounts of δ -tocopherol were detected, and no β -tocopherol was found in the caneberry seeds. ^b Values are on a μ mol TE/g oil basis. One gram of oil is equal to 1.1 mL.

Table 4. Means and Standard Errors of ORAC, Total Phenolic Content, and Ellagic Acid Content in Caneberry Whole Seeds

source	ORAC ^a (μ M TE/g seed)	total phenolics ^b (mg GA/g seed)	ellagic acid (mg/g seed)
red raspberry	539.7 \pm 22.9	58.1 \pm 0.58	8.7 \pm 0.5
black raspberry	150.7 \pm 7.50	44.6 \pm 0.52	6.7 \pm 0.5
boysenberry	255.0 \pm 25.2	49.1 \pm 0.24	30.1 \pm 2.4
Marion blackberry	146.0 \pm 14.7	50.3 \pm 0.72	32.3 \pm 2.1
evergreen blackberry	199.7 \pm 8.40	54.4 \pm 0.92	21.2 \pm 1.9

^a Values are on a μ mol TE/g seed basis. ^b Values represent mg gallic acid equivalents (GA)/g seed.

Ellagic acid and ellagitannins have been reported to be abundant in caneberries (23). We estimated the total ellagic acid content in the seeds after acid hydrolysis so as to include ellagitannins in the estimation. Red and black raspberry seeds contained 8.7 and 6.7 mg/g seed, respectively (Table 4). Boysenberry, Marion blackberry, and evergreen blackberry contained 30, 32, and 21 mg/g ellagic acid. The total ellagic acid and ORAC values were not related ($R^2 = 0.16$; $P = 0.50$).

To detect other possible constituents of the total phenolics, we used reverse-phase HPLC on berry seed aqueous extracts. Two main groups of peaks were developed (Figure 1). The peak at 61 min in all five chromatograms corresponded to an ellagic acid standard, and smaller peaks in the vicinity are likely ellagic acid derivatives (24). A second group of peaks occurred in the vicinity of 34 min. After alkaline hydrolysis, the group of peaks at 34 min in red raspberry seed disappeared, and the ellagic acid peak at 61 min increased over 5-fold (Figure 1F). Hydrolysis was similarly applied to evergreen blackberry, with similar results (data not shown). Thus, alkaline hydrolysis suggested that the major peaks detected in aqueous extract by HPLC correspond to ellagitannins and free ellagic acid. No peaks corresponded to our standards of catechin, epicatechin, chlorogenic acid, or gallic acid, and there was no evidence of anthocyanins.

DISCUSSION

The caneberry seed protein levels of 6–7% are much less than the 20–40% in common oilseeds (25). The amount of lysine in the caneberry seeds was between 0.29 and 0.32% dry matter, while maize grain is between 0.21 and 0.28% (26). For threonine, methionine, and tryptophan, amino acids commonly limiting in animal diets, the caneberry seeds had lower percentages than maize and other grains (26). Oil comprised 11–19% of the seed with hexane extraction, consistent with earlier findings in red raspberry (4–6). With the expeller press, 5–12% of seed weight was oil (data not shown). These oil percentages are lower than most oilseeds but similar to extraction rates of other specialty oils such as grapeseed (27).

The Pacific Northwest grows the majority of caneberries in the United States, but only the portion utilized in seedless processing provides the current supply of seed. It is estimated that over 180 000 kg of berry seed is available in Oregon and Washington (3). At 10% oil yield of an estimated 180 000 kg of caneberry seed per year, 18 000 kg of oil could be extracted. This amount is small, for example, in comparison to 1000–1200 tons of evening primrose oil annually exported from China (28). In 2002, the United States produced approximately 16% of the world's caneberries (2). Thus, much more seed could be available on a global scale, but the worldwide percentage of caneberries that are processed to remove seeds is unavailable.

Caneberry oils had two distinguishing features from common vegetable oils. Vegetable oils usually contain between 6 and

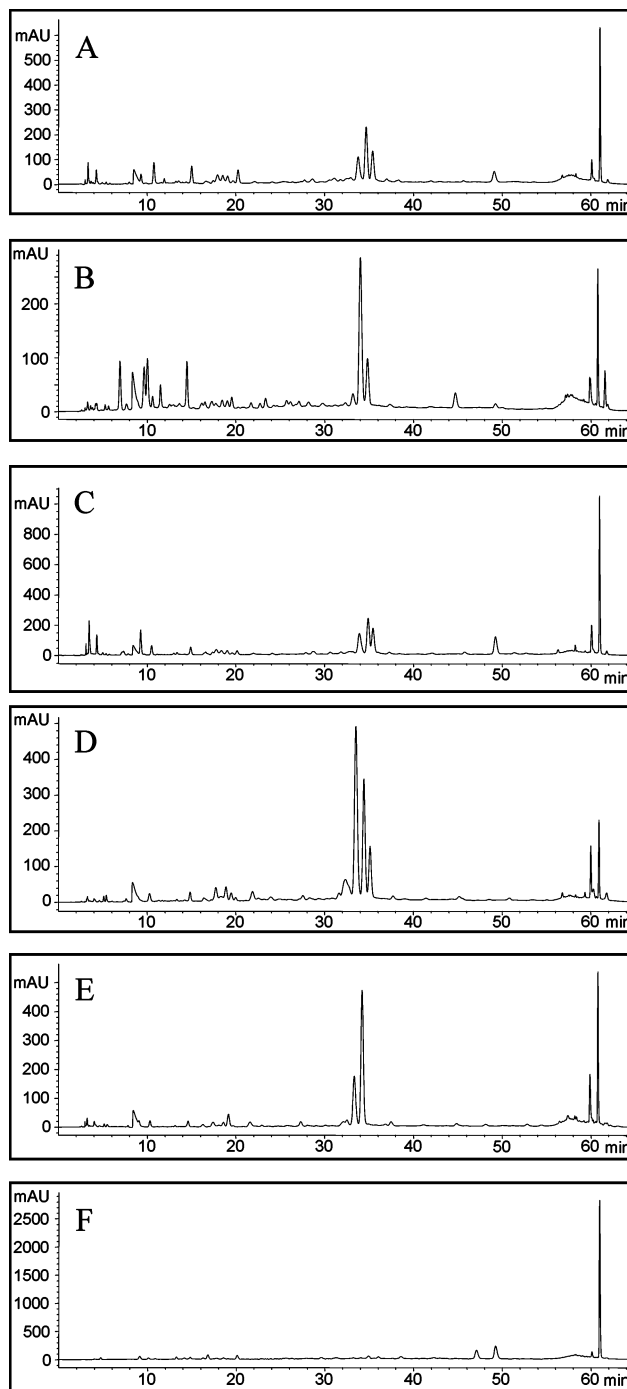


Figure 1. HPLC chromatograms of berry seed aqueous extracts reported with a photodiode array detector at 260 nm. (A) Red raspberry, (B) black raspberry, (C) boysenberry, (D) Marion blackberry, (E) evergreen blackberry, and (F) red raspberry after alkaline hydrolysis.

15% saturated fatty acids (25, 29, 30). The saturated fatty acid levels of the caneberry seed oils were between 3 and 5.5%, although 7.8% was present in evergreen blackberry. Caneberry oils also contained between 15 and 31% α -linolenic acid; the blackberry oils had the least, boysenberry had an intermediary amount, and the raspberry oils had the most. α -Linolenic acid and its derivatives eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have received attention for their effects on brain and retina function (31), their suppressive effects on coronary heart disease (32), their antiinflammatory properties (33), and their involvement in infant development (34, 35). The major commercial source of α -linolenic acid is flaxseed, while

canola and soybean oils contain much smaller amounts (25). The range of 15–31% α -linolenic acid in caneberry seed oils is high relative to the vegetable oils and more similar to other berry oils such as black currant, blueberry, and cranberry seeds (6–8).

Linoleic and α -linolenic acids constituted the majority of the polyunsaturated fats in the caneberry seed oils, between 74 and 84% of the oil. In humans, these fatty acids are substrates for β -oxidation, carbon recycling, or conversion in the liver to EPA and DHA (10). Linoleic acid can compete with α -linolenic acid for the same metabolism enzymes (36). The abundance of linoleic and α -linolenic acids in tissue lipids can thus be affected by the choice of ingested fats (37, 38), and the proportion of ω -6/ ω -3 fatty acids has been proposed as an accurate method of predicting coronary heart disease (32). Most vegetable oils have ω -6/ ω -3 ratios in excess of 6/1, whereas ratios of 2/1 are currently considered beneficial (32, 39). Caneberry seed oil ratios range from 1.7/1 in the two raspberries to 4/1 in Marion blackberries.

The caneberry seeds contained between 10 and 33 mg total tocopherols/100 g seed. This range exceeds the amounts in many cereal grains and nuts (10). Tocopherols have been reported to inhibit lipid oxidation in ORAC assays (19) and in radical-trapping antioxidant parameter assays (21), and we detected ORAC values for expeller-pressed caneberry oils in the range of 52–120 μ M TE/g oil. We are not aware of other lipophilic ORAC studies on plant oils. The ORAC values support the presence of antioxidants in the lipids, but we found no correlation between tocopherol levels and oil ORAC values. We assume that all of the tocopherols are recovered in the oil; nevertheless, dividing the total tocopherol amounts by the percent oil still did not allow significant correlation between the two values. Caneberry oil ORAC values may also be due to the presence of other lipophilic antioxidants, like tocotrienols or carotenoids.

Caneberry whole seeds had high ORAC values: comparable to green tea (40) and blueberry fruits (41) and in excess of many other fruits and vegetables (40, 42). Previously, ORAC values were obtained from whole fruits of the same five caneberry varieties reported in this study (22), but our seed ORAC values did not parallel those of the whole fruits. Whereas the darker colored caneberry fruits had the highest ORAC values (22), presumably due to the antioxidant contributions of anthocyanins, red raspberry seed had the highest ORAC value of the five species that we tested.

The major hydrophilic molecules that we observed in HPLC chromatograms of seeds were ellagitannins and free ellagic acid. This is consistent with previous reports of high amounts of both ellagitannins and free ellagic acid in red raspberry, 88% of which reside in the seed (13). Mullen et al. analyzed red raspberry whole fruits and identified abundant sanguin H-6 and lambertianin C and several additional ellagitannins in smaller amounts (43). It is interesting that the two blackberry species and the boysenberry hybrid contained appreciably more total ellagic acid than the two raspberry species.

Each of the caneberry species had its own distinguishing compositional profile, and as a group, they could be defined by their abundance of α -linolenic acid, ellagic acid, and antioxidant capacity.

ABBREVIATIONS USED

AOCS, American Oil Chemists' Society; AOAC, Association of Analytical Chemists; ORAC, oxygen radical absorbance capacity; trolox, 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic

acid; HPLC, high-performance liquid chromatography; PUFA, polyunsaturated fatty acid; DHA, docohexanoic acid; EPA, eicosapentaenoic acid.

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LITERATURE CITED

- (1) *Noncitrus Fruits and Nuts 2003 Summary*; National Agricultural Statistics Service, United States Department of Agriculture: Washington DC, 2003.
- (2) *FAOSTAT Data, 2003*. Food and Agriculture Organization of the United Nations, <http://faostat.fao.org/faostat/collections?subset=agriculture>, last accessed August 2004.
- (3) Oregon Blackberry and Raspberry Commission. *Assessing Market Opportunities for Raspberry and Blackberry Seeds*; Oregon Blackberry and Raspberry Commission: Corvallis, OR, 2003.
- (4) Oomah, B. D.; Ladet, S.; Godfrey, D. V.; Liang, J.; Girard, B. Characteristics of raspberry (*Rubus idaeus* L.) seed oil. *Food Chem.* **2000**, *69*, 187–193.
- (5) Winton, A. L.; Winton, K. B. *The Structure and Composition of Foods*; John Wiley and Sons: New York, 1935; Vol. II, pp 618–634.
- (6) Johansson, A.; Laakso, P.; Kallio, H. Characterization of seed oils of wild, edible Finnish berries. *Z. Lebensm. Unters. Forsch. A* **1997**, *204*, 300–307.
- (7) Parker, T. D.; Adams, D. A.; Zhou, K.; Harris, M.; Yu, L. Fatty acid composition and oxidative stability of cold-pressed edible seed oils. *Food Chem. Toxicol.* **2003**, *68*, 1240.
- (8) Barre, D. E. Potential of evening primrose, borage, black currant, and fungal oils in human health. *Ann. Nutr. Metab.* **2001**, *45*, 47–57.
- (9) Takahata, K.; Monobe, K.; Tada, M.; Weber, P. C. The benefits and risks of n-3 polyunsaturated fatty acids. *Biosci., Biotechnol., Biochem.* **1998**, *62*, 2079–2085.
- (10) Sinclair, A. J.; Attar-Bashi, N. M.; Li, D. What is the role of α -linolenic acid for mammals? *Lipids* **2002**, *37*, 1113–1123.
- (11) Sheppard, A. J.; Pennington, J. A. T.; Weihrauch, J. L. Analysis and distribution of vitamin E in vegetable oils and foods. In *Vitamin E in Health and Disease*; Packer, L., Fuehs, J., Eds.; Marcel-Dekker: New York, 1993; pp 9–31.
- (12) Bramley, P. M.; Elmadfa, I.; Kafatos, A.; Kelly, F. J.; Manios, Y.; Roxborough, H. E.; Schuch, W.; Sheehy, P. J. A.; Wagner, K.-H. Vitamin E. *J. Agric. Food Chem.* **2000**, *80*, 913–938.
- (13) Daniel, E. M.; Krupnick, A. S.; Heur, Y.; Blinzler, J. A.; Nims, R. W.; Stoner, G. D. Extraction, stability, and quantitation of ellagic acid in various fruits and nuts. *J. Food Comp.* **1989**, *2*, 338–349.
- (14) Stoner, G. D.; Morse, M. A. Isothiocyanates and plant polyphenols as inhibitors of lung and esophageal cancer. *Cancer Lett.* **1997**, *114*, 113–119.
- (15) Xue, H.; Aziz, R. M.; Sun, N.; Cassady, J. M.; Kamendulis, L. M.; Xu, Y.; Stoner, G. D.; Klaunig, J. E. Inhibition of cellular transformation by berry extracts. *Carcinogenesis* **2001**, *22*, 351–356.
- (16) Slinkard, K.; Singleton, V. L. Total phenol analysis: automation and comparison with manual methods. *Am. J. Enol. Vitic.* **1977**, *28*, 49–55.
- (17) Ou, B.; Hampsch-Woodill, M.; Prior, R. L. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *J. Agric. Food Chem.* **2001**, *49*, 4619–4626.
- (18) Huang, D.; Ou, B.; Hampsch-Woodill, M.; Flanagan, J. A.; Prior, R. L. High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. *J. Agric. Food Chem.* **2002**, *50*, 4437–4444.

- (19) Huang, D.; Ou, B.; Hampsch-Woodill, M.; Flanagan, J. A.; Deemer, E. K. Development and validation of oxygen radical absorbance capacity assay for lipophilic antioxidants using randomly methylated cyclodextrin as the solubility enhancer. *J. Agric. Food Chem.* **2002**, *50*, 1815–1821.
- (20) Podda, M.; Weber, C.; Traber, M. G.; Packer, L. Simultaneous determination of tissue tocopherols, tocotrienols, ubiquinol, and ubiquinones. *J. Lipid Res.* **1996**, *37*, 893–901.
- (21) Cabrini, L.; Barzanti, V.; Cipollone, M.; Fiorentini, D.; Grossi, G.; Tolomelli, B.; Zamboni, L.; Landi, L. Antioxidants and total peroxyl radical-trapping ability of olive and seed oils. *J. Agric. Food Chem.* **2001**, *49*, 6026–6032.
- (22) Wada, L.; Ou, B. Antioxidant activity and phenolic content of Oregon caneberrries. *J. Agric. Food Chem.* **2002**, *50*, 3495–3500.
- (23) Clifford, M. N.; Scalbert, A. Ellagitannins—Nature, occurrence, and dietary burden. *J. Sci. Food Agric.* **2000**, *80*, 1118–1125.
- (24) Mullen, W.; McGinn, J.; Lean, M. E. J.; MacLean, M. R.; Gardner, P.; Duthie, G. G.; Yokota, T.; Crozier, A. Ellagitannins, flavonoids, and other phenolics in red raspberries and their contribution to antioxidant capacity and vasorelaxation properties. *J. Agric. Food Chem.* **2002**, *50*, 5191–5197.
- (25) Norton, G. Nature and biosynthesis of storage proteins. In *Oil Crops of the World*; Robbelen, G., Downey, R. K., and Ashri, A., Eds.; McGraw-Hill: New York, 1989; pp 165–191.
- (26) *United States–Canadian Tables of Feed Composition*, 3rd revision; National Academy Press: Washington, DC, 1982; pp 112–128.
- (27) Kamel, B. S.; Dawson, H.; Kakuda, Y. Characteristics and composition of melon and grape seed oils and cakes. *J. Am. Oil Chem. Soc.* **1985**, *62*, 881–883.
- (28) Deng, Y.; Hua, H.; Li, J.; Lapinskas, P. Studies on the cultivation and uses of evening primrose (*Oenothera* spp.) in China. *Econ. Bot.* **2004**, *55*, 83–92.
- (29) Goffman, F. D.; Bohme, T. Relationship between fatty acid profile and vitamin E content in maize hybrids (*Zea mays* L.). *J. Agric. Food Chem.* **2001**, *49*, 4990–4994.
- (30) Kamal-Eldin, A.; Yanishlieva, N. V. N-3 fatty acids for human nutrition: stability considerations. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 825–836.
- (31) Fernstrom, J. D. Effects of dietary polyunsaturated fatty acids on neuronal function. *Lipids* **1999**, *34*, 161–167.
- (32) Okuyama, H.; Fujii, Y.; Ikemoto, A. n-6/n-3 ratio of dietary fatty acids rather than hypercholesterolemia as the major risk factor for atherosclerosis and coronary heart disease. *J. Health Sci.* **2000**, *46*, 157–177.
- (33) KanKaanpaa, P.; Sutas, Y.; Salminen, S.; Lichtenstein, A.; Isolauri, E. Dietary fatty acids and allergy. *Ann. Med.* **1999**, *31*, 282–287.
- (34) Xiang, M.; Zetterstrom, R. Relation between polyunsaturated fatty acids and growth. *Acta Paediatr.* **1999**, *88*, 78–82.
- (35) Gibson, R. A.; Makrides, M. The role of long chain polyunsaturated fatty acids (LCPUFA) in neonatal nutrition. *Acta Paediatr.* **1998**, *87*, 1017–1022.
- (36) Seppanen-Laakso, T.; Laakso, I.; Hiltunen, R. Analysis of fatty acids by gas chromatography, and its relevance to research on health and nutrition. *Anal. Chim. Acta* **2002**, *465*, 39–62.
- (37) Biagi, P.; Bordoni, A.; Lorenzini, A.; Horrobin, D. F.; Hrelia, S. Essential fatty acid metabolism in long-term primary cultures of rat cardiomyocytes: A beneficial effect of n-6:n-3 fatty acids supplementation. *Mech. Ageing Dev.* **1999**, *107*, 181–195.
- (38) Keys, A.; Anderson, J. T.; Grande, F. Prediction of serum-cholesterol responses of man to changes in fats in the diet. *Lancet* **1957**, *273*, 959–966.
- (39) Simopoulos, A. P. Essential fatty acids in health and chronic disease. *Am. J. Clin. Nutr.* **1999**, *70* (Suppl.), 560S–569S.
- (40) Cao, G.; Sofic, E.; Prior, R. L. Antioxidant capacity of tea and common vegetables. *J. Agric. Food Chem.* **1996**, *44*, 3426–3431.
- (41) Prior, R. L.; Cao, G.; Martin, A.; Sofic, E.; McEwen, J.; O'Brien, C.; Lischner, N.; Ehlenfeldt, M.; Kalt, W.; Krewer, G.; Mainland, C. M. Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of *Vaccinium* species. *J. Agric. Food Chem.* **1998**, *46*, 2686–2693.
- (42) Wang, H.; Cao, G.; Prior, R. L. Total antioxidant capacity of fruits. *J. Agric. Food Chem.* **1996**, *44*, 701–705.
- (43) Mullen, W.; Yokota, T.; Lean, M. E. J.; Crozier, A. Analysis of ellagitannins and conjugates of ellagic acid and quercetin in raspberry fruits by LC-MSn. *Phytochemistry* **2003**, *64*, 617–624.

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