平成２６年７月１６日

公益社団法人　全国消費生活相談員協会
理事長　吉川　萬里子　殿

サニーヘルス株式会社
代表取締役　西村　峯満

回　答　書

貴協会より平成２６年７月４日付でご連絡のありました件につきご回答いたします。

記

１：平成２６年５月３１日付日本経済新聞夕刊掲載広告の表示内容について

ご指摘のありました新聞広告につきましては、急遽空いた広告枠への出稿だったため、広告代理店保管の過去原稿を流用いたしました。その際の手違いで、４月以前に使用していた旧い表記の広告原稿を使用してしまいました。今後はHP同様の修正をいたします。

２：弊社HP内テロップの表示内容について

ご指摘のありましたテロップにつきましては、一連の修正作業の中で、修正が漏れていたものです。ただちに４月のご指摘内容に則り表現を改めました。

３：「ボイセンベリーのエラグ酸量がブルーベリーの300倍」であることの根拠を示す出典について

ご要望のありました出典につきましては、原文と日本語訳を別紙にて提出いたしますので、ご確認のほどよろしくお願い申し上げます。

なお、「J. Agric. Food Chem. Vol.50,No.8.2002,」（以下Ａ）がブルーベリーのエラグ酸含有量を、「J. Agric. Food Chem. Vol.50,No.12.2002,」（以下Ｂ）がボイセンベリーのエラグ酸含有量を示すデータとなり、ＡとＢの比較により数字を算出しております。

Ａの P.2434 よりブルーベリーのエラグ酸含有量は 0.19mg/100g 生果実重量、Ｂの P.3499 のグラフより読み取るとボイセンベリーのエラグ酸含有量は約 70mg/100g 生果実重量となり、70÷0.19≒368 で、ボイセンベリーはブルーベリーの300倍以上のエラグ酸を含むという根拠としております。

以上

（本件に関する連絡先）
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J. Agric. Food Chem., Vol. 50, No.8 2002

『Phenolic Compounds and Antioxidant Capacity of Georgia-Grown Blueberries and Blackberries.(ジョージア州産ブルーベリーとブラックベリーのフェノール化合物と抗酸化能)』

p.2434

≪結果≫　フェノール酸とフラボノイド

表2はフェノール酸とフラボノイドの含量を示している。

エラグ酸について、ラビットアイブルーベリーではほとんどの品種で検出出来なかった。しかしながら、ブラックベリーからは高濃度のエラグ酸を検出した(Choctaw種:33.81mg/100g 生果実重量、Kiowa 種:30.01mg/100g 生果実重量)。また、サザンハイハイブリッシュブルーベリーからは 0.75～6.65 ㎎/100g(生果実重量)検出された。

【表2】ブルーベリーとブラックベリーのフェノール酸とフラボノイドの量

		フェノール酸(㎎/100g　生果実重量)					
	品種(収穫条件)	没食子酸	P-ハイドロキシベンゼン酸	コーヒー酸	p-クマリン酸	フェルラ酸	エラグ酸
ラビットアイブルーベリー	Austin	1.53±0.31	nd	nd	nd	3.57±0.59	0.22±0.05
	Briteblue	2.83±0.96	nd	nd	7.91±1.65	4.51±0.59	nd
	Brightwell	4.03±1.24	nd	nd	4.37±0.94	3.02±0.64	6.02±0.71
	Climax (6月上旬)	4.04±0.47	nd	2.40±0.35	3.78±0.92	5.14±1.06	1.12±0.15
	Climax (かんがい栽培：6月下旬)	nd	103.67±9.95	nd	nd	nd	nd
	Climax (かんがい無し：6月下旬)	nd	nd	6.32±0.10	15.78±3.09	nd	nd
	FL81-11	1.57±0.18	nd	nd	nd	5.09±0.03	nd
	Premier	4.23±0.90	nd	nd	7.48±0.13	4.34±0.04	nd
	FL81-156	1.55±0.06	nd	nd	nd	3.22±0.46	0.19±0.00
	T460	3.42±0.48	nd	nd	5.13±0.90	3.17±0.04	nd
	Tifblue	248.9±69.21	nd	nd	nd	16.97±0.06	nd
	Woodard	4.01±0.38	nd	4.07±0.76	10.16±1.51	4.10±0.07	nd
サザンハイブリッシュブルーベリー	FL86-19	4.55±1.04	nd	nd	4.75±0.83	3.45±0.33	nd
	TH161	2.72±0.64	nd	nd	7.15±1.50	4.16±0.64	4.45±0.17
	TH440	1.95±0.06	nd	nd	4.62±0.39	3.63±0.59	6.65±0.69
	TH442	4.76±0.89	nd	3.33±0.13	6.27±0.36	nd	0.75±0.10
	Sharpblue	2.83±0.83	nd	3.00±1.88	2.40±0.62	nd	2.68±0.32
ブラックベリー	Choctaw	6.42±0.32	nd	1.38±0.83	2.08±0.29	3.51±0.59	33.81±2.62
	Kiowa	4.12±0.35	nd	3.64±0.26	0.40±0.01	2.99±0.22	30.01±1.23

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Phenolic Compounds and Antioxidant Capacity of Georgia-Grown Blueberries and Blackberries

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Blueberries and blackberries grown at various locations in Georgia were collected and analyzed for flavonoids, total anthocyanins, total polyphenols, and Trolox-equivalent antioxidant capacity (TEAC). Each sample was analyzed for phenolic acids (gallic acid, *p*-hydroxybenzoic acid, caffeic acid, ferulic acid, and ellagic acid) and flavonoids (catechin, epicatechin, myricetin, quercetin, and kaempferol). A high-performance liquid chromatographic (HPLC) method with photodiode array detection was used for analysis. Compounds were analyzed as aglycons after acid hydrolysis with 1.2 M HCl. Identification of each compound was based on retention time and UV spectra by comparison with pure commercial standards. Phenolic acids ranged from 0.19 to 258.90 mg/100 g fresh weight (FW), and flavonoids ranged from 2.50 to 387.48 mg/100 g FW. Total polyphenols ranged from 261.95 to 929.62 mg/100 g FW, and total anthocyanins ranged from 12.70 to 197.34 mg/100 g FW. TEAC values varied from 8.11 to a maximum of 38.29 μM/g FW. A linear relationship was observed between TEAC values and total polyphenols or total anthocyanins. The data indicate that blueberries and blackberries are rich sources of antioxidants.

KEYWORDS: Anthocyanins; antioxidant capacity; blackberries; blueberries; flavonoids; HPLC diode-array; phenolic acids; total polyphenols

INTRODUCTION

Phenolics are naturally occurring secondary metabolites from plants. They are present in fruits, vegetables, leaves, nuts, seeds, flowers, and barks. These compounds are an integral part of the human diet and are also taken intentionally as medicinal preparations. Since ancient times, plant preparations have been used by man to deal with common health problems. However, the importance of these compounds as health-promoting and disease-preventing substances has recently been realized through scientific investigations. Phenolic compounds are considered as nonnutrient biologically active compounds (*1*). The functionality of these compounds is expressed through their action as an inhibitor or an activator for a large variety of mammalian enzyme systems, and as metal chelators and scavenger of free oxygen radicals (*2*−*4*). Oxygen free radicals are involved in many pathological conditions such as atherosclerosis, cancer, and chronic inflammation (*5*). Phenolics interfere with the pathways that regulate cell division and proliferation, platelet aggregation, detoxification, and inflammatory and immune response (*6*). Among these phenolic substances, flavonoids, and in particular anthocyanins, are of interest because of their high occurrence in foods, especially in fruits, vegetables, and green leafy vegetables including green tea. Flavonoids are known to reduce coronary heart disease (*7*), and they have anticancer (*8*, *9*) and antioxidant properties (*10*).

In view of growing interest in these compounds, there is a need to identify and quantify these important compounds in fruits and vegetables. Some of the compounds are present in many fruits but others are specific for a particular kind of fruit or vegetable. Furthermore, within the same fruit type, the growing season, variety, environmental and climatic conditions, plant disease, soil type, geographic locations, and even maturity, seem to influence the concentration of phenolic compounds.

Because blueberries are grown in large scale in Georgia and they are currently being promoted as a rich source of antioxidants, the present work focused on further characterization of berries grown in Georgia as possible sources of phenolics for functional foods application. We analyzed different varieties of blueberries and blackberries for total anthocyanins, total polyphenols, and Trolox-equivalent antioxidant capacity. The phenolics analyzed were gallic acid, *p*-hydroxybenzoic acid, caffeic acid, *p*-coumaric acid, ferulic acid, ellagic acid, (+)-catechin, (−)-epicatechin, myricetin, quercetin, and kaempferol.

MATERIALS AND METHODS

Chemicals. Pure standards were purchased from Sigma (St. Louis, MO) and Fluka (Milwaukee, WI). Standards were dissolved in methanol as follows: gallic acid, 2.1 mg; *p*-hydroxybenzoic acid, 2.1 mg; (+)-

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Table 1. Solvent Gradient Elution Program

time (min)	A 1% formic acid in 70:30 water/methanol (%)	B methanol (%)	C 1% formic acid in water (%)	flow (mL/min)
0.00	0.0	0.0	100	1.3
5.00	0.0	0.0	100	1.3
5.01	50.0	0.0	50.0	1.3
10.00	85.0	15.0	0.0	1.0
10.01	50.0	5.0	45.0	1.0
20.00	85.0	15.0	0.0	1.0
25.00	85.0	15.0	0.0	1.0
60.00	45.0	55.0	0.0	1.0
60.01	0.0	100.0	0.0	1.0
65.00	0.0	100.0	0.0	1.0
65.01	50.0	0.0	50.0	1.0
75.00	50.0	0.0	50.0	1.0

catechin, 2.5 mg; caffeic acid, 2 mg; (−)-epicatechin, 2.9 mg; *p*-coumaric acid, 2.3 mg; ferulic acid, 2.4 mg; ellagic acid, 2.4 mg; myricetin, 2.3 mg; quercetin, 2.1 mg; and kaempferol 2.2 mg, all per 10 mL. Working solutions were prepared each day by appropriate dilution with methanol. 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid), and ascorbic acid were purchased from Fluka (Milwaukee, WI). Methanol and water (HPLC grade), formic acid, and hydrochloric acid (analytical grade) were purchased from Fisher Scientific (Norcross, GA). Ascorbic acid was procured from BASF Corporation (Parsippany, NJ).

Sample Collection. Blueberry and blackberry samples were collected from Chula, Alapaha, and Attapulgus (Georgia) in June 2000. Varieties of berries collected were rabbiteye blueberries (*Vaccinium ashei* Reade) cultivars: Austin, Brightblue, Brightwell, Climax (Alapaha), Climax (irrigated, Attapulgus), Climax (nonirrigated, Attapulgus, drought-strickened), FL 80-11, Premier, FL 81-156, T 460, Tifblue, and Woodard; Southern highbush blueberries (*Vaccinium corymbosum L.* Hybrids) cultivars: FL 86-19, TH 161, TH 440, TH 442, and Sharpblue; and blackberries (*Rubus L.*) cultivars: Choctaw and Kiowa. All cultivars were grown with irrigation or under conditions of adequate rainfall, except the nonirrigated Climax which were drought strickened. Samples were frozen, transported to the University of Georgia, and stored at −80 °C for further use.

Extraction and Hydrolysis. A 10-g portion of frozen whole fruit sample was ground to paste with mortar and pestle in the presence of 100 mg of ascorbic acid, 500 mg of washed sand, and 10 mL of 6 M HCl. Volume was made to 50 mL with methanol (final concentration of 1.2 M HCl). The flask was wrapped with aluminum foil and flushed with nitrogen for 5 min. The deoxygenated sample was refluxed at 95 °C for 2 h to hydrolyze the flavonoid glycosides to aglycons. The hydrolyzed sample was cooled in dark and filtered through a 0.2-micron syringe nylon filter. A 20-μL aliquot of filtered sample was injected into the HPLC for analysis.

HPLC Analysis. HPLC was performed with a Hewlett-Packard (Avondale, PA), model 1090 liquid chromatograph with quaternary pumps and a diode array UV-visible detector (*11*−*13*) coupled to a HP ChemStation. A Phenomenex (Torrance, CA) Prodigy 5-μ, ODS-2, RP C_{18} column (250 × 4.6 mm) protected by guard column was the stationary phase. Gradient of mobile phase (A) water/methanol (70:30 vol/vol) with 1% formic acid, (B) methanol, and (C) 1% formic acid in water with a flow rate of 1−1.3 mL/min was used as shown in **Table 1**. UV spectra were recorded from 220 to 450 nm at a rate of 1.00 spectrum/1.28 s and a resolution of 2 nm with a bandwidth of 4 nm and reference wavelength in off mode.

Blueberries have been analyzed by HPLC with photodiode array detection (*11*−*13*) using a C_{18} reverse-phase column in acidic pH. However, our attempt to follow those methods resulted in an unsatisfactory performance due to baseline drift. To improve the separation of these compounds in berries we modified the method of Justesen et al. (*14*) as described in **Table 1**. Separation of the following compounds

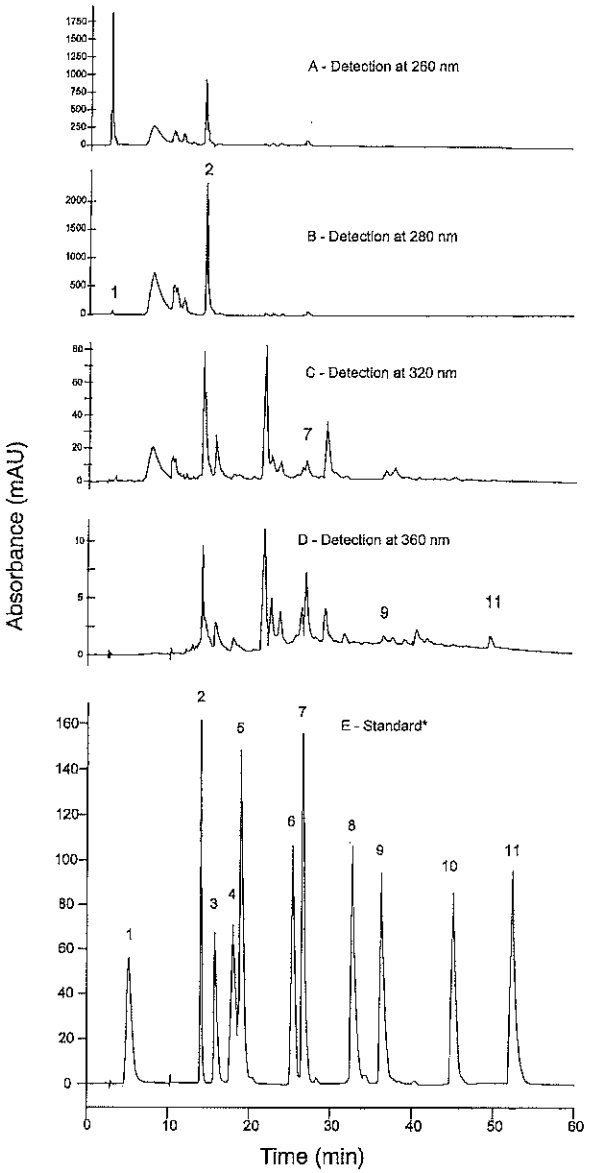

Figure 1. HPLC chromatogram of rabbiteye blueberry sample FL 80-11 detected at 260 nm (A), 280 nm (B), 320 nm (C), and 360 nm (D). The chromatogram of standard is presented as E. *The standards were detected at 280 nm from 0 to 19 min, 320 nm from 19 to 30 min, 260 nm from 30 to 35 min, and 360 nm from 35 to 60 min. Compounds: (1) gallic acid, (2) (+)-catechin, (3) *p*-hydroxybenzoic acid, (4) (−)-epicatechin, (5) caffeic acid, (6) *p*-coumaric acid, (7) ferulic acid, (8) ellagic acid, (9) myricetin, (10) quercetin, and (11) kaempferol.

occurred in the order listed: gallic acid, catechin, *p*-hydroxybenzoic acid, epicatechin, caffeic acid, *p*-coumaric acid, ferulic acid, ellagic acid, myricetin, quercetin, and kaempferol. Satisfactory separation was achieved and the separation of epicatechin and caffeic acid was much closer than that of any other compounds. The HPLC chromatogram of separated standard compounds and a blueberry sample is shown in **Figure 1**. However, the presence of many other unknown compounds in the real sample made the chromatogram too crowded; but the unwanted peaks could be selectively suppressed by scanning at specific predetermined wavelengths of 260, 280, 320, and 360 nm. Use of narrower bandwidth of 4 nm in each signal improved the peak sharpness.

Quantitation. Quantification was performed based on external standards of known concentrations. Peak areas were used to quantify

the compounds in the sample. Calibration curves of the standards ranging from 20 to 240 ng/mL were used with good linearity and R^2 values exceeding 0.99 (peak areas vs concentration).

Analysis of Total Anthocyanins, Total Polyphenols, and Antioxidant Capacity. *Extraction.* The method of Prior et al. (*15*) was adopted with minor modification for the extraction of phenolics. Briefly, 1 g of frozen sample was pasted with mortar and pestle in 10 mL of 4% acetic acid in acetonitrile, and the final volume was made up to 25 mL with the same solution. Contents were shaken at 200 rpm for 1 h at 30 °C in a Gyratory water bath shaker. The extract was filtered with 0.2-micron syringe nylon filter before analysis.

Estimation of Anthocyanins. Total anthocyanin content of berries was estimated on a UV−visible spectrophotometer (Shimadzu UV-1601, Norcross, GA) by the pH-differential method using two buffer systems − potassium chloride buffer, pH 1.0 (0.025 M) and sodium acetate buffer, pH 4.5 (0.4 M). A diluted sample of 0.2 mL (to give optical density in the range of 0.1−1.2 at 510 nm) was mixed with 1.8 mL of corresponding buffer and read against a blank at 510 and 700 nm. Absorbance was calculated as

$$A = (A_{510nm} - A_{700nm})_{pH1.0} - (A_{510nm} - A_{700nm})_{pH4.5}$$

Monomeric anthocyanin pigment concentration in the extract was calculated as cyanidin-3-glucoside (*16*).

$$\text{Monomeric anthocyanin pigment (mg/L)} = A \times MW \times DF \times 1000/(\epsilon \times 1)$$

where A = absorbance, MW = molecular weight (449.2); DF = dilution factor, ϵ = molar absorptivity (26,900). The final concentration of anthocyanins (mg/100 g) was calculated based on total volume of extract and weight of sample.

Estimation of Total Polyphenols. Total polyphenols were estimated colorimetrically using the Folin−Ciocalteau method (*17*). Extracted samples were filtered through a 0.2-μm nylon syringe filter. A sample aliquot of 200 μL was added to 800 μL of water, 5 mL of 0.2 N Folin−Ciocalteau reagent, and 4 mL of saturated sodium carbonate solution (75 g/L) and mixed in a cyclomixer. The absorbance was measured at 765 nm with a Shimadzu UV−Visible spectrophotometer after incubation for 2 h at room temperature. Quantification was based on the standard curve generated with 100, 200, 300, and 400 mg/L of gallic acid.

Assay of Antioxidant Capacity. Antioxidant capacity was performed on the Shimadzu UV−Visible spectrophotometer in a kinetic mode based on the method of Re et al. (*18*). Briefly, $ABTS^{\bullet+}$ radical cation was produced by reacting 7 mM of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 2.45 mM potassium persulfate after incubation at room temperature in dark for 16 h. The $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.70 ± 0.1 at 734 nm. Filtered sample was diluted with ethanol so as to give 20−80% inhibition of the blank absorbance with 20 μL of sample. A 980 μL aliquot of $ABTS^{\bullet+}$ solution (absorbance of 0.70 ± 0.1) was read at 734 nm for a minute; after exactly 1 min, 20 μL of the sample was added and mixed thoroughly. Absorbance was continuously taken at every 6 s up to 7 min. Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid, a vitamin E analogue) standards of final concentration 0−15 μM in ethanol were prepared and assayed under the same condition. The Trolox-equivalent antioxidant capacity (TEAC) of the sample was calculated based on the inhibition exerted by standard Trolox solution at 6 min.

RESULTS

Phenolic Acids and Flavonoids. The contents of individual phenolic acids and flavonoids are reported in **Table 2**. The highest concentration of gallic acid was found in rabbiteye blueberry, Tifblue at 258.90 mg/100 g fresh weight [FW], compared to southern highbush blueberries and blackberries. Other varieties within rabbiteye blueberries have comparable gallic acid content. Climax (irrigated, late June) and Climax (nonirrigated, late June) had no detectable amounts of gallic acid. *p*-Hydroxybenzoic acid was found only in rabbiteye blueberry, Climax-irrigated, at 103.67 mg/100 g FW. Southern highbush blueberries and blackberries had no detectable amounts of *p*-hydroxybenzoic acid. Caffeic acid was up to 6.32 mg/100 g FW in rabbiteye blueberries, which is slightly higher than southern highbush blueberries and blackberries containing up to 3.33 and 3.64 mg/100 g FW, respectively. The lowest concentration (1.38 mg/100 g FW) of caffeic acid was found in Choctaw cultivar of blackberry. *p*-Coumaric acid was found in all three varieties. Rabbiteye blueberry, Climax (nonirrigated) had highest *p*-coumaric acid of 15.78 mg/100 g among the three varieties. Blackberries had the lowest content of *p*-coumaric acid, 0.40 mg/100 FW. The majority of the cultivars within rabbiteye blueberries contain *p*-coumaric acid, except Austin, Climax-irrigated, FL-80-11, Fl-81-156, and Tifblue. Southern highbush blueberry cultivars contain *p*-coumaric acid in the range of 2.40−7.15 mg/100 g FW. The highest amount of ferulic acid was found in rabbiteye blueberries, Tifblue at 16.97 mg/100 g, and moderate amounts of up to 4.16 and 3.51 mg/100 g FW were found in southern highbush blueberries and blackberries, respectively. Rabbiteye blueberry cultivars Climax-irrigated and Climax-nonirrigated, and southern highbush blueberry cultivars TH 442 and Sharpblue, did not have any detectable amounts of ferulic acid. We did not detect ellagic acid in many cultivars of rabbiteye blueberries. However, high concentrations of ellagic acid were found in blackberries, Choctaw (33.81 mg/100 g FW) and Kiowa (30.01 mg/100 g FW). Southern highbush blueberries contained 0.75−6.65 mg/100 g FW ellagic acid, whereas rabbiteye blueberries contained 0.19−33.81 mg/100 g FW ellagic acid.

Catechin was the major flavonoid, with concentrations of up to 387.48 mg/100 g FW, followed by epicatechin at up to 129.51 mg/100 g FW. Flavonoids myricetin, quercetin, and kaempferol contents were less than 14.60 mg/100 g FW among all three varieties. The highest concentration of catechin was found in rabbiteye blueberries, Austin (387.48 mg/100 g FW), followed by blackberries, Choctaw (312.86 mg/100 g FW). Southern highbush blueberries contained up to 29.28 mg catechin/100 g FW. The presence of high concentrations of catechins suggests the possible occurrence of more polar dimeric and oligomeric proanthocyanidins with important biological activities. Epicatechin was found only in rabbiteye blueberries and ranged from 34 to 129.51 mg/100 g FW, with the highest concentration foundin Briteblue (129.51 mg/100 g FW); moderate concentrations were found in Climax (Alapaha), Climax-irrigated (Attapulgus), T460, and Woodard. Southern highbush blueberries and blackberries did not have any detectable amount of epicatechin. The majority of the cultivars contained myricetin in the range of 6.68−9.99 mg/100 g FW. Kiowa (blackberry) had the highest myricetin concentration of 9.99 mg, and the lowest concentration was found in Climax (6.68 mg/100 g FW). Southern highbush blueberries had up to 6.98 mg/100 g FW. Myricetin content was found to be higher than that previously reported by Häkkinen et al. (*12, 13*), which was 23−26 mg/kg FW. Southern highbush blueberry FL 86-19 had the highest concentration of quercetin, 14.60 mg/100 g FW, followed by Climax (Attapulgus) at 9.97 mg/100 g FW in rabbiteye blueberries. These values are slightly lower than previous reports of 17−24 mg/kg FW (*12*), but they are in good agreement with Häkkinen et al. (*13*), who reported 10.5−16.0 mg/100 g FW. No quercetin was detected in blackberries or the following cultivars of rabbiteye blueberries: Austin, Climax-irrigated, FL 80-11, FL 81-156, and Tifblue. Kaempferol contents of rabbiteye blueberries and southern highbush blueberries were up to 3.72 and 3.17 mg/

Table 2. Individual Phenolic Acids and Flavonoids in Blueberries and Blackberries (values are averages of triplicate analyses)

	phenolic acids (mg/100 g freshweight)						flavonoids (mg/100 g freshweight)				
cultivar and sample location	gallic acid	*p*-hydroxy benzoic acid	caffeic acid	*p*-coumaric acid	ferulicacid	ellagic acid	catechin	epicatechin	myricetin	quercetin	kaempferol
				Rabbiteye blueberries (*Vaccinium ashei* Reade)							
Austin[a]	1.53 ± 0.31	nd[d]	nd	nd	3.57 ± 0.59	0.22 ± 0.05	387.48 ± 13.90	nd	nd	nd	2.60 ± 0.07
Briteblue[a]	2.83 ± 0.97	nd	nd	7.91 ± 1.65	4.51 ± 0.59	nd	28.04 ± 16.75	129.51 ± 1.91	6.69 ± 0.02	5.82 ± 0.09	2.59 ± 0.04
Brightwell[a]	4.03 ± 1.24	nd	nd	4.37 ± 0.94	3.02 ± 0.64	6.02 ± 0.71	15.51 ± 4.27	nd	7.05 ± 0.45	6.81 ± 0.68	2.68 ± 0.17
Climax (early June)[a]	4.04 ± 0.47	nd	2.40 ± 0.35	3.78 ± 0.92	5.14 ± 1.06	1.12 ± 0.15	17.51 ± 6.57	57.68 ± 4.37	6.68 ± 0.06	6.20 ± 0.23	2.50± 0.12
Climax (irrigated, late June)	nd	103.67 ± 9.95	nd	nd	nd	nd	17.43 ± 7.33	nd	nd	nd	3.07 ± 0.06
Climax (nonirrigated, late June)[b]	nd	nd	6.32 ± 0.10	15.78 ± 3.09	nd	nd	34.75 ± 1.51	34.23 ± 0.30	nd	9.97 ± 0.88	3.21 ± 0.18
FL 80-11[a]	1.57 ± 0.18	nd	nd	nd	5.09 ± 0.03	nd	277.83 ± 18.23	nd	6.73 ± 0.34	nd	2.70 ± 0.06
Premier[a]	4.23 ± 0.90	nd	nd	7.48 ± 0.13	4.34 ± 0.04	nd	17.26 ± 0.07	nd	7.07 ± 0.07	6.10 ± 0.14	3.72 ± 0.04
FL 81-156[c]	1.55 ± 0.06	nd	nd	nd	3.22 ± 0.46	0.19 ± 0.00	246.66 ± 55.67	nd	8.62 ± 0.09	nd	nd
T 460[a]	3.42 ± 0.48	nd	nd	5.13 ± 0.90	3.17 ± 0.04	nd	14.53 ± 4.50	37.89 ± 4.61	6.69 ± 0.1	7.59 ± 0.61	2.51 ± 0.04
Tifblue[b]	258.90± 69.21	nd	nd	nd	16.97 ± 0.06	nd	107.00 ± 22.26	nd	nd	nd	nd
Woodard[a]	4.01 ± 0.38	nd	4.07± 0.76	10.16 ± 1.51	4.10 ± 0.07	nd	17.69 ± 0.22	48.66 ± 1.23	6.81 ± 0.08	5.88 ± 0.22	2.69 ± 0.08
				Southern highbush blueberries (*Vaccinium corymbosum L.* Hybrids)							
FL 86-19[c]	4.55 ± 1.04	nd	nd	4.75 ± 0.83	3.45 ± 0.33	nd	11.66 ± 0.74	nd	6.72 ± 0.12	14.60±0.38	2.52 ± 0.03
TH 161[a]	2.72 ± 0.64	nd	nd	7.15 ± 1.50	4.16 ± 0.64	4.45 ± 0.17	21.43 ± 2.84	nd	6.91 ± 0.34	9.85 ± 1.56	2.59 ± 0.20
TH 440[a]	1.95 ± 0.06	nd	nd	4.62 ± 0.39	3.63 ± 0.59	6.65 ± 0.69	25.25 ± 2.13	nd	nd	12.03 ± 0.28	2.88 ± 0.10
TH 442[a]	4.76 ± 0.89	nd	3.33 ± 0.13	6.27 ± 0.36	nd	0.75 ± 0.10	29.28 ± 1.75	nd	6.98 ± 0.37	10.28 ± 0.65	3.17 ± 0.62
Sharpblue	2.83 ± 0.83	nd	3.00 ± 1.88	2.40 ± 0.62	nd	2.68 ± 0.32	9.87 ± 0.00	nd	nd	9.70 ± 4.23	3.13 ± 0.01
				Blackberries (*Rubus L.*) cultivars							
Choctaw[c]	6.42 ± 0.32	nd	1.38 ± 0.83	2.08 ± 0.29	3.51 ± 0.59	33.81 ± 2.62	312.86 ± 8.71	nd	nd	nd	nd
Kiowa[c]	4.12 ± 0.35	nd	3.64 ± 0.26	0.40 ± 0.01	2.99 ± 0.22	30.01 ± 1.23	265.75 ± 11.46	nd	9.99 ± 1.05	nd	nd

[a] Alapaha, GA. [b] Attapulgus, GA. [c] Chula, GA. [d] nd = Not detected.

Table 3. Total Anthocyanins, Total Polyphenolics, and TEAC Values of Blueberries and Blackberries (values are averages of triplicates)

cultivar and location	total anthocyanins[d] (mg/100 g FW)	total polyphenolics (mg/100 g FW)	TEAC[e] μM/g FW
	Rabbiteye blueberries (*Vaccinium ashei* Reade)		
Austin[a]	178.15 ± 11.62	669.01 ± 6.57	29.52 ± 3.42
Brightblue[a]	16.37 ± 0.39	929.62 ± 20.40	26.74 ± 1.96
Brightwell[a]	87.38 ± 8.10	386.86 ± 10.64	29.81± 1.72
Climax[a] (early June)	105.21 ± 5.16	288.00 ± 9.16	22.65 ± 3.12
Climax (irrigated, late June)[b]	197.34 ± 5.69	641.07 ± 21.33	30.06 ± 2.67
Climax (nonirrigated, late June)[b]	99.33 ± 2.81	270.02 ± 15.42	19.73 ± 0.98
FL 80-11[a]	171.92 ± 4.36	911.78 ± 8.70	24.99 ± 2.60
Premier[a]	157.31 ± 1.82	522.13 ± 25.25	38.29 ± 2.89
FL 81-156[b]	111.47 ± 3.68	603.36 ± 21.19	24.87 ± 3.45
T 460[a]	12.70 ± 0.33	437.37 ± 20.27	21.19 ± 2.32
Tifblue[b]	108.62 ± 1.90	391.57 ± 10.17	29.66 ± 3.10
Woodard[a]	116.85 ± 1.05	622.89 ± 13.74	33.76 ± 3.81
average	113.55 ± 58.10	556.14 ± 216.87	27.60 ± 5.33
	Southern highbush blueberries (*Vaccinium corymbosum L.* Hybrids)		
FL 86-19[c]	35.47 ± 1.37	261.95 ± 62.93	8.11 ± 1.81
TH 161[a]	87.63 ± 9.36	287.87 ± 56.97	8.62 ± 1.39
TH 440[a]	53.49 ± 5.48	327.93 ± 60.65	10.37 ± 1.38
TH 442[a]	114.06 ± 15.55	585.34 ± 3.57	26.45 ± 3.60
Sharpblue	129.93 ± 4.10	533.32 ± 47.55	20.60 ± 2.89
average	84.12 ± 39.72	399.28 ± 149.12	14.83 ± 8.24
	Blackberries (*Rubus L.*) cultivars		
Choctaw[c]	110.52 ± 3.04	555.21 ± 68.36	18.04 ± 4.16
Kiowa[c]	122.66 ± 4.73	417.84 ± 25.80	2.65 ± 1.22
average	116.59 ± 8.58	486.53 ± 97.13	20.35 ± 3.25

[a] Alapaha, GA. [b] Attapulgus, GA. [c] Chula, GA. [d] Total anthocyanins were expressed as cyanidin-3-glucoside equivalents. [e] TEAC: Trolox-equivalent antioxidant capacity at 6 min.

100 g FW, respectively. These values are slightly higher than previous reports of 0—0.6 mg/100 g FW (*12*). Blackberries do not have any detectable amount of kaempferol.

Overall, our results are in good agreement with those reported in the literature (*19—22*) except for gallic acid. Unlike these authors, our findings show high concentrations of gallic acid in many of the cultivars. The concentrations of caffeic acid, ellagic acid, and myricetin in blueberries are in good agreement with the previous findings (*23*). Nevertheless, the concentrations of kaempferol, *p*-coumaric acid, and ferulic acid were slightly higher than the reported values of 0.6, 0.7, and 0.8 mg, respectively (*23*). The presence of quercetin and kaempferol in highbush blueberries is in good agreement with previous findings by Kader et al. (*23*). The large standard deviation in some of the results underscores the difficulty in obtaining evenly matured fruit samples for analysis.

Total Anthocyanins. Anthocyanins content of individual cultivars are shown in **Table 3**. The average total anthocyanin contents among rabbiteye blueberries, southern highbush blueberries, and blackberries are 113.55, 84.12, and 116.59 mg/100 g FW, respectively. Blackberries had the highest concentration and southern highbush blueberries had the least. Among rabbiteye blueberry varieties, Climax (irrigated-late June harvest) had the highest concentration of anthocyanins at 197.34 mg/100 g FW. On a very dry site, irrigation appeared to double the anthocyanin content of Climax. Sharpblue southern highbush blueberries and Kiowa in blackberries had the highest concentrations of 129.93 and 122.66 mg anthocyanins/100 g FW, respectively. These values are in good agreement with, and within the range of, those in previous reports (*15*).

Total Polyphenols. All flavonoids, anthocyanins, and nonflavonoid phenolic compounds are estimated in this parameter. Because only a few of the whole spectra of compounds could be identified and quantified, total polyphenols (TPP) estimates the whole amount of phenolics present in samples. **Table 3** presents the TPP of all samples. Rabbiteye blueberries had the highest average concentration of TPP (556.14 mg) and southern highbush blueberries had the lowest (399.28 mg/100 g FW). Blackberries had an average 486.53 mg TPP/100 g FW. Briteblue from rabbiteye blueberries had the highest amount of TPP at 929.62 mg/100 g FW, followed by FL-80-11 at 911.78/100 g FW, among all cultivars. Climax (nonirrigated) was found to contain the least amount (270.02 mg TPP/100 g FW) among rabbiteye blueberries. Overall, the lowest concentration of TPP was found in the southern highbush FL86-19 variety at 261.95 mg/100 g FW.

Antioxidant Capacity. Assessing the capacity of a compound to scavenge $ABTS^{\bullet+}$ radicals in terms of Trolox equivalent is known as Trolox-equivalent antioxidant capacity (TEAC) as first reported by Miller et al. (*26*). Various phytochemical components, including the flavonoids, phenylpropanoids, and phenolic acids are known to be responsible for antioxidant capacity in fruits and vegetables (*27*). Among the cultivars assayed, the values were found to be in the range of 8.11 to 38.29 μM TEAC/g of FW (**Table 3**). The average TEAC values for rabbiteye blueberries, southern highbush blueberries, and blackberries were 27.60, 14.83, and 20.35 μM TEAC/g FW, respectively. The antioxidant capacity may be related to the content of phenolic compounds in these samples. Higher content of TPP reflected higher TEAC values, and reduction in TPP decreased the TEAC value (*26*). The Premier cultivar from rabbiteye blueberries gave the highest TEAC value of 38.29 μM TEAC/g FW. Nonirrigated drought-strickened Climax gave the lowest antioxidant capacity of 19.73 μM TEAC/g FW among the rabbiteye blueberries. TH 442 had the highest TEAC value of 26.45, and FL-86-19 had the lowest (8.11 TEAC) value in southern highbush blueberries. Blackberry cultivars, Choctaw

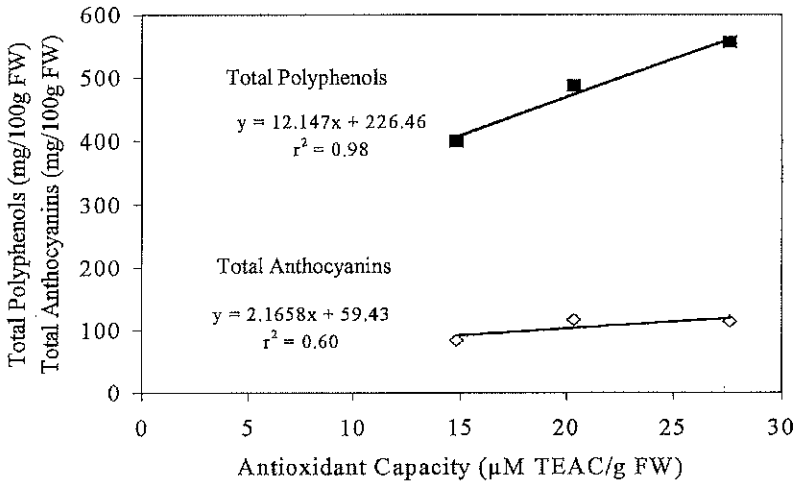

Figure 2. Correlation between total polyphenols (*y*-axis), $r^2 = 0.98$, and total anthocyanins (*y*-axis), $r^2 = 0.60$, to TEAC value (*x*-axis). Average values for rabbiteye blueberries, southern highbush blueberries, and blackberries were used for the plots.

and Kiowa, had moderate TEAC values of 18.04 and 22.65 μM TEAC/g FW, respectively, in comparison with those of other cultivars.

Catechin was present in all types of berries analyzed. The majority of the berries contained gallic acid, ferulic acid, myricetin, quercetin, and kaempferol. Anthocyanins were present from 12 to 197 mg/100 g FW and total polyphenols varied from 261 to 929 mg/100 g FW. TEAC values of berries analyzed were in the range of 8 to 38 μM/g FW. These variations may be due to the variety, type of the cultivars, maturity, and soil conditions in Georgia.

The correlation between TEAC and total polyphenols or total anthocyanins contents of different blueberry and blackberry samples are presented in **Figure 2**. The average values of TEAC showed positive correlation with average values of total anthocyanins and total polyphenols. A linear relationship was observed between TEAC and total polyphenols or total anthocyanins. The correlation coefficient, r^2, is 0.98 for total polyphenols and 0.60 for total anthocyanins. These values indicate that the antioxidant capacity is strongly related to total polyphenols and moderately related to total anthocyanins. Similar correlation was reported with oxygen radical absorbance capacity (ORAC) for other cultivars of southern highbush and lowbush blueberries with total polyphenols $r^2 = 0.85$, and $r^2 = 0.77$ for total anthocyanins (*15*). However, the correlation of TEAC values to total anthocyanins or total polyphenols for individual varieties deviated much. The correlation coefficient between TEAC values and total anthocyanins was 0.20; and between TEAC and total polyphenols was 0.05, for rabbiteye blueberries. Southern highbush blueberry TEAC values showed strong correlation with total anthocyanins, $r^2 = 0.65$ and total polyphenols, $r^2 = 0.98$. Blackberry TEAC values were correlated with total anthocyanins, $r^2 = 1$; and total polyphenols, $r^2 = 1$. These differences can be explained by the wide range of values in certain varieties (**Table 3**) and/or by the lack of enough cultivars in the case of blackberries.

Our findings are in general agreement with the work of Prior et al. (*15*) regarding total anthocyanins, total polyphenols, and antioxidant capacity of rabbiteye blueberries. The average content of total anthocyanins, total polyphenols, and antioxidant capacity of rabbiteye blueberries were higher than than those of southern highbush. Blackberry averages were similar to those of rabbiteye blueberries. Georgia grown blueberries and blackberries are a good source of antioxidants that can be used in foods and nutritional supplement formulations.

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『Antiocidant Actibity and Phenolic Content of Oregon Caneberries. (オレゴン州のケインベリー(エバーグリーンブラックベリー、マリオンベリー、ボイセンベリー、レッドラズベリー、ブラックラズベリー)の抗酸化能とフェノール含量)』

p.3499

≪結果≫

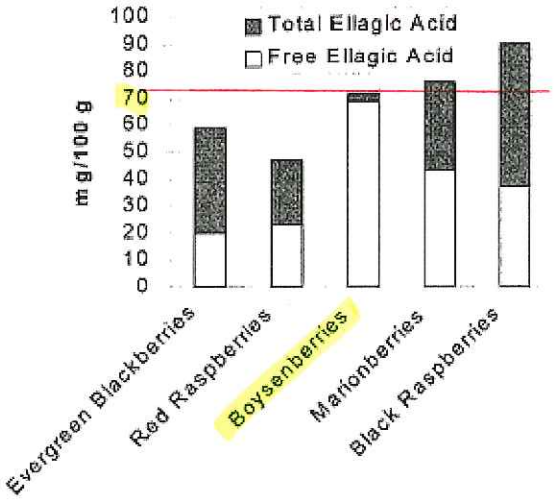
各果実のエラグ酸の総量を測定した(図 3)。一番少ない果実はレッドラズベリー(47 mg/100g)で、一番多い果実はブラックラズベリー(90mg/100g)であった。そのうち、遊離エラグ酸の量は大凡 40～50%程度であった。一方、ボイセンベリーのエラグ酸はほとんどが遊離体で存在していた。エラグ酸は食品中では、分子量の高いエラジタンニンという形で存在している。それゆえ、食品中のエラグ酸総量を測定するには、加水分解して遊離エラグ酸の形にする必要があった。

また、エラグ酸は抗発がん物質として注目されていたにも関わらず、公的な食品中のエラグ酸量のデータは限られた食品でしかなかった。ストロベリーやラズベリー、トゲ無しブラックベリーはエラグ酸の豊富な果実として報告されていた。本研究では、加えて、ブラックベリーやボイセンベリーもエラグ酸が豊富であることが分かった。

つまり、ボイセンベリーはエラグ酸総量が多いだけでなく、利用可能なエラグ酸が多い果実である。

【図 3】５つのベリーのエラグ酸総量と遊離エラグ酸の量

■総エラグ酸量　　□遊離エラグ酸量



左から

- エバーグリーンブラックベリー
- レッドラズベリー
- ボイセンベリー
- マリオンベリー
- ブラックラズベリー

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Antioxidant Activity and Phenolic Content of Oregon Caneberries

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Five types of caneberries [evergreen blackberries (*Rubus laciniatus*), marionberries (*Rubus ursinus*), boysenberries (*Rubus ursinus* × *idaeus*), red raspberries (*Rubus idaeus*), and black raspberries (*Rubus occidentalis*)] were analyzed for antioxidant activity by measuring their oxygen radical absorbance capacity (ORAC). In addition, the berries were analyzed for total phenolics, anthocyanins, procyanidins, and ellagic acid content. All of the berries had high ORAC activity ranging from 24 to 77.2 μmol of Trolox equiv/g of fresh berries. Anthocyanin content ranged from 0.65 to 5.89 mg/g, and phenolics ranged from 4.95 to 9.8 mg/g. Black raspberries had the highest ORAC and anthocyanin and phenolic contents. Only red raspberries had detectable amounts of procyanidin oligomers (monomer, dimers, and trimers). All berries had high levels of ellagic acid (47−90 mg/g), but boysenberries had the highest level prior to hydrolysis. The results from this study indicate that these caneberries were high in antioxidant activity and were rich sources of anthocyanins and phenolics.

KEYWORDS: Caneberries; blackberries; raspberries; boysenberries; ORAC; anthocyanins; phenolics; procyanidin; ellagic acid

INTRODUCTION

High intakes of fruits and vegetables have been associated with lower incidences of chronic diseases such as cancer and heart disease (*1*, *2*). In addition to the vitamins and minerals known to be present in fruits and vegetables, phytochemicals such as flavonoids and other phenolics may contribute to this protective effect. Many of these phytochemicals have antioxidant activity and may help protect cells against the oxidative damage caused by free radicals.

Recently there has been an increasing amount of attention given to the health benefits of consuming berries such as blueberries (*3*, *4*). Blueberries contain anthocyanins, the flavonoid pigment responsible for their red to bluish hue. Anthocyanins exhibit antioxidant activity (*5*) and inhibit low-density lipoprotein (LDL) oxidation (*6*). They have also been shown to have vasoprotective and antiinflammatory activity (*7*, *8*). Anthocyanin-rich extracts from European berries such as the bilberry (*Vaccinium myrtillus*) have been sold commercially to treat microcirculation disease and maintain normal vascular permeability (*9*).

Prior et al. have reported that blueberries possess considerably high antioxidant activity that is attributed in part to their anthocyanin content (*3*). Heinonen et al. used two oxidation models, human LDL and lecithin liposomes, to study the antioxidant activity of various berries including red raspberries and black raspberries. They found the berries to possess antioxidant activity, although the degree of activity and relative ranking varied with the model used (*10*). Others have assessed the antioxidant activity of berries including black and red raspberries by measuring the superoxide scavenging activity (*11*). Recently, Wang and Lin (*12*) measured the oxygen radical absorbance capacity (ORAC) of red raspberries, black raspberries, and blackberries and found these berries to have high ORAC levels.

Some berries such as strawberries and black raspberries have been identified as sources of the phenolic compound ellagic acid, which has been demonstrated to have potential cancer chemopreventive activity (*13*). Other berries such as blueberries and cranberries have been identified as sources of procyanidins. Procyanidins are a group of flavonoids composed of flavan-3-ol monomers linked together into polymers of various lengths. Oligomeric procyanidins have been shown to increase plasma antioxidant capacity (*14*) and inhibit atherosclerosis in rabbits (*15*). The ability of blueberries and cranberries to inhibit *Escherichia coli* adhesion and prevent urinary tract infection has been attributed to their procyanidin content (*16*).

The purpose of this study was to study a particular group of berries known as caneberries. Caneberries are a group of berries that grow on leafy canes in temperate regions of the world. The best known commercial caneberries are the red raspberry (*Rubus idaeus*), black raspberry (*Rubus occidentalis*), marionberry (*Rubus ursinus*), evergreen blackberry (*Rubus laciniatus*), and boysenberry (*Rubus ursinus* × *idaeus*). In the United States, caneberries were used centuries ago by Native Americans and settlers for food, as dye for clothing, and for medicinal purposes. In the 19th century cultivated production began in the Pacific Northwest, which is still the leading area of caneberry production (*17*).

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Caneberries are considered to be nutritious, are low in fat, and provide a good source of dietary fiber, vitamin C, and potassium (*17*). To learn more about the antioxidant activity and phytochemical composition of caneberries grown in Oregon, samples were analyzed for ORAC and phenolic, anthocyanin, procyanidin, and ellagic acid contents.

MATERIALS AND METHODS

Chemicals. β-Phycoerythrin, gallic acid, catechin, rutin, isoquercitrin, epicatechin gallate, quercitrin, myricetin, quercetin, and kaempferol were purchased from Sigma Chemical Co. (St. Louis, MO). 6-Hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox) was obtained from Aldrich (Milwaukee, WI). 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was obtained from Wako Chemicals USA, Inc. (Richmond, VA). Methanol and acetonitrile (HPLC grade) were from Fisher Scientific (Springfield, NJ).

Berries. Five species of caneberries were evaluated in this study. These included red raspberries (*R. idaeus*), black raspberries (*R. occidentalis*), marionberries (*R. ursinus*), evergreen blackberries (*R. laciniatus*), and boysenberries (*R. ursinus* × *idaeus*). All berries were grown near Salem, OR. Only berries that were ripe by commercial standards (no green or overripe berries) were harvested and collected from a central processing plant close to the berry farms. Approximately 500 g of each berry was shipped frozen and received overnight. Samples were kept frozen until analysis. At least three determinations were performed for each analysis, and the average was reported.

ORAC Assay. The 500 g sample of each berry was initially ground in a mechanical mill. Then 0.5 g was accurately weighed, and 20 mL of acetone/water (50:50 v/v) extraction solvent was added. The mixture was shaken for 1 h at room temperature on an orbital shaker. On the basis of data from recovery studies we had previously performed, this amount of time was adequate to extract most phenolic compounds. The extracts were centrifuged at 5900 rpm, and the supernatant was ready for analysis. The ORAC assay is based on a procedure of Cao et al. (*18*) and modified for the COBAS FARA II spectrofluorometric centrifugal analyzer (Roche Diagnostic System Inc., Branchburg, NJ) (*19*). Fluorescence is measured at 565 nm with the excitation wavelength at 540 nm. AAPH is used as the source for the peroxyl radical, which is generated as a result of the spontaneous decomposition of AAPH at 37 °C. β-Phycoerythrin is the chosen target protein; its loss of fluorescence is an indication of the extent of damage from its reaction with the peroxyl radical. The protective effect of the antioxidants is measured by assessing the longer fluorescence time/intensity area under the curve of the sample compared to that of the blank in which no antioxidant compounds are present. Trolox, a water soluble analogue of vitamin E, was used as the calibration standard. Fluorescence readings are taken every 2 min for up to 70 min after the addition of AAPH. The ORAC results are calculated on the basis of the calibration curve obtained in each run and reported as micromoles of Trolox equivalents (TE) per gram of fresh weight (FW) or dry matter (DM). Dry matter was determined after lyophilization.

Anthocyanins. *Sample Preparation.* Five grams of the milled whole berries was ground again in a high-speed mill. After grinding, 0.5 g samples were extracted with methanol acidified with 0.1% HCl. The extractions were performed on an orbital shaker operated at 400 rpm at room temperature for 1 h. This has proved to be adequate for complete extraction. The samples were then centrifuged at 5900 rpm for 15 min. The supernatant was recovered and filtered through a 0.45 μm cellulose syringe filter before analysis.

Total Anthocyanin Assay. The total anthocyanins were estimated by a pH differential method (*20*). Absorbance was measured in a COBAS FARA II spectrofluorometric centrifugal analyzer at 510 nm and at 700 nm in buffer at pH 1.00 and pH 4.5, using $A = (A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}$ with a molar extinction coefficient of cyanidin 3-glucoside of 29600. Results were expressed as milligrams of cyanidin 3-glucoside equivalent per gram of fresh weight or dry matter.

LC-MS Structural Analysis. The structural information of individual anthocyanins was obtained by liquid chromatography coupled with ion trap mass spectrometry (LC-MS^n). The system used for LC-MS analysis was a Finnigan MAT LCQ ion trap mass spectrometer (ThermoFinnigan, San Jose, CA) equipped with an HP 1100 binary system consisting of an autosampler and diode array detector (Hewlett-Packard, Palo Alto, CA) set at 550 nm. The separation of anthocyanin was performed using a Phenomenex (Torrance, CA) 5 μm Phenyl Hexyl column (250 × 4.6 mm). 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. The ionization parameters for the mass spectrometer were optimized using constant infusion of cyanidin 3-glucoside to the ion source. The heated capillary and voltage were maintained at 175 °C and 2 kV, respectively. The full-scan mass spectra from *m/z* 100 to 1000 were collected. Tandem mass spectrometry was performed using helium as the collision gas, and the collision energy was set at 25%. All mass spectrometry data were acquired in the positive ionization mode.

Phenolics. *Sample Preparation.* Approximately 15 g of ground berries was weighed into a 50 mL polyethylene centrifuge tube with 20 mL of methanol. The sample was extracted for 1 h at room temperature on an orbital shaker operated at 400 rpm and then centrifuged at 5900 rpm, and the supernatant was immediately analyzed. For ellagic acid (EA), 15 g of the berries was extracted by methanol at 100 °C for 24 h (containing free EA). The extract was then evaporated to dryness and hydrolyzed in 2 N trifluoroacetic acid in methanol at 100 °C for 2 h (containing total EA).

Total Phenolic Assay. Total phenolics content was determined according to the Folin−Ciocalteu procedure on a COBAS FARA II centrifugal analyzer using gallic acid as a standard (*21*). The instrument was operated in the spectrophotometric mode measuring the absorption of 750 nm. The total phenolic content was expressed as gallic acid equivalents in milligrams per 100 g.

LC-MS Structural Analysis. A validated LC-MS method was utilized for structural analysis of phenolic compounds. HPLC conditions were identical to those used for anthocyanin analysis as mentioned above except that the diode array detector was set at 278 nm. The ionization parameters for the mass spectrometer were optimized using constant infusion of rutin to the ion source. The heated capillary and voltage were maintained at 200 °C and 3.5 kV, respectively. The collision energy was set at 30%. All mass spectrometry data were acquired in the positive ionization mode. Catechin, epicatechin, gallate, rutin, isoquercitrin, quercitrin, myricetin, quercetin, kaempferol, chlorogenic acid, benzoic acid, caffeic acid, ellagic acid, ferulic acid, and coumaric acid were used as standards. The identities of individual compounds were confirmed by the retention time and molecular weight obtained by LCQ mass detector.

Procyanidins. *Sample Preparation.* Five grams of each berry was accurately weighed into a 50 mL polyethylene centrifuge tube with 20 mL of extraction solvent containing acetone, water, and acetic acid (70:29.5:0.5 v/v). The mixture of solvent and sample was vortexed, sonicated for 5 min in a water bath at 50 °C, and allowed to extract for 30 min. The mixture was centrifuged at 5900 rpm for 15 min. The resulting supernatant was rotary evaporated at 50 °C under partial vacuum, and the residue was diluted to 5 mL with deionized water. The procyanidin fraction was isolated using SPE columns, which were wet packed with 5 g of Sephadex LH-20 hydrated in 25 mL of water. An aliquot of 5 mL of each of the extracted samples was loaded onto the column. Each column was eluted with 45 mL of 20% methanol/water (v/v) to remove sugars and phenolic acids, followed by 40 mL of 60% methanol/water (v/v) to elute the flavonols and anthocyanins, and finally with 90 mL of 100% methanol for elution of the procyanidins. Other types of samples that have higher concentrations of procyanidins may require more rigorous procedures. However, this procedure was sufficient to elute the amount of procyanidins in these berry samples. The 100% methanol fraction was concentrated by rotary evaporation, and the concentrated material was diluted to a final volume of 5 mL with 100% methanol.

LC-MS Structural Analysis. The LC-MS method was based on that of Adamson and co-workers (*22*). Fluorescence detection was recorded at an excitation wavelength of 276 nm and an emission wavelength of 316 nm. Normal phase separations of the procyanidin oligomers were performed on a Phenomenex (Torrance, CA) 5 μm Luna Silica (3.0 × 250 nm) column at 37 °C with a 5 μL injection volume. The binary

Table 1. Antioxidant Activity (ORAC) and Anthocyanin and Phenolic Contents of Berries

species	ORAC[a,b] (μmol of TE/g)	phenolics[b,c] (mg/g)	anthocyanins[c,d] (mg/g)
evergreen blackberries	27.5 ± 2.6 (175)	4.95 ± 0.13 (30.94)	0.91 ± 0.02 (5.69)
red raspberries	24.0 ± 1.8 (171)	5.17 ± 0.12 (36.93)	0.65 ± 0.03 (4.64)
boysenberries	42.2 ± 2.5 (350)	5.99 ± 0.16 (49.92)	1.31 ± 0.01 (10.92)
marionberries	28.0 ± 2.9 (215)	5.83 ± 0.15 (44.85)	1.55 ± 0.02 (11.92)
black raspberries	77.2 ± 2.5 (453)	9.80 ± 0.10 (57.65)	5.89 ± 0.04 (34.65)

[a] Expressed as micromoles of Trolox equivalents per gram of fresh fruit. [b] Data in parentheses expressed per gram of dry matter. [c] Concentration based upon gallic acid as standard expressed per gram of fresh weight. [d] Concentration based upon cyanidin 3-glucoside as standard expressed per gram of fresh weight.

Table 2. Percent Contribution of Individual Anthocyanins to Total Anthocyanins

anthocyanin	evergreen blackberries (%)	red raspberries (%)	boysen-berries (%)	marion-berries (%)	black raspberries (%)
cyanidin 3-(6′-*p*-coumaryl)glucoside-5-glucoside			56.27[a]		
cyanidin 3,5-diglucoside		89.25			
cyanidin 3-(6′-*p*-coumaryl)sambubioside					22.00
cyanidin 3-(6′-*p*-coumaryl)glucoside				94.86[b]	77.00
cyanidin 3-glucoside	80.43	10.75	43.73		
cyanidin 3-arabinoside	10.19				
cyanidin 3-(6′-malonyl)glucoside	6.06			1.27	
unknown	3.40			3.87	1.00

[a] Coelutes with cyanidin 3,5-diglucoside (minor amount). [b] Coelutes with cyanidin 3-glucoside (minor amount).

mobile phase consisted of (A) dichloromethane, methanol, water, and acetic acid (82:14:2:2, v/v) and (B) methanol, water, and acetic acid (96:2:2, v/v). The gradient method started at 1 mL/min from 0 to 17.6% B in 30 min, followed by 17.6−30.7% B in 15 min, and then 30.7−87.8% in 15 min. In all cases, the columns were re-equilibrated between injections with the equivalent of 25 mL of the initial mobile phase. Catechin standards were prepared and analyzed to establish a response calibration curve from which to calculate the concentration of procyanidins in the samples. The structures of the procyanidins were confirmed by molecular weights obtained by LCQ.

For the LCQ mass spectrometer, an ionization reagent (1.5 M NaOH) was added at a rate of 0.05 mL/min through a tee before the mass detector and was delivered using a secondary HPLC pump. Catechin was used as a calibration standard for tuning the mass detector. The heated capillary and voltage were maintained at 275 °C and 2 kV, respectively. The full-scan mass spectra from *m/z* 100 to 2000 were collected. The collision energy was set at 30%. All mass spectrometry data were acquired in the negative ionization mode.

RESULTS AND DISCUSSION

The antioxidant activity as measured by ORAC ranged from 24 μmol of Trolox equivalents (TE)/g in the fresh red raspberries to 77 μmol of TE/g in the fresh black raspberries (171−453 μmol of TE/g of dry matter) (**Table 1**). These values are high when compared to previous values reported for fruits and vegetables (*23*, *24*) and are as high or higher than ORAC values found in blueberries (*3*). The ORAC values of our marionberries and evergreen blackberries (28 μmol of TE/g) were similar to the range of 20.3−24.6 μmol of TE/g found in blackberries by Wang and Lin (*12*), who measured ORAC in blackberries and raspberries grown at the Beltsville Agricultural Research Center in Beltsville, MD. They also reported ORAC values for red raspberries of 15.9−20 μmol of TE/g, which was similar to our value of 24 μmol of TE/g. The ORAC for boysenberries (42 μmol of TE/g) was higher than that seen in either red raspberries or blackberries. We do not know of any other ORAC data on boysenberries. The ORAC of black raspberries was much higher than the levels in the other caneberries. The greatest difference between analyzed and reported values was seen with black raspberries. Wang and Lin (*12*) reported an ORAC value of 28.2 μmol of TE/g, whereas our analysis found a high ORAC of 77 μmol of TE/g. This difference may be due in part to the difference in cultivar. Wang and Lin used black raspberries of the Jewel cultivar, whereas the cultivar of our black raspberries was Munger. In addition, our analysis was performed on the whole berry including seeds, whereas Wang and Lin used only the juice expressed from the berries.

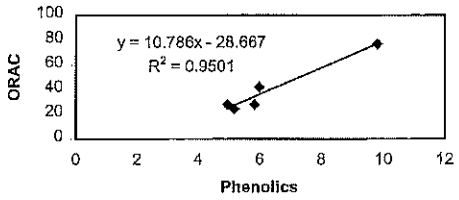

Figure 1. Relationship between ORAC (μmol of TE/g) (*Y*) and phenolics (mg/g) in caneberries.

Total phenolics ranged from 4.95 mg/g in evergreen blackberries to 9.80 mg/g in the black raspberries (30.94−57.65 mg/g of dry matter) (**Table 1**). As illustrated in **Figure 1**, a good linear relationship was found between ORAC and total phenolic content ($r^2 = 0.9501$), implying that the antioxidant activity of caneberries is largely due to the presence of phenolic compounds. The phenolic contents of evergreen blackberries, red raspberries, boysenberries, and marionberries were similar (4.95−5.99 mg/g), whereas black raspberries (9.8 mg/g) had higher levels than the other berries.

Black raspberries also had the highest level of anthocyanins, with 5.89 mg/g (34.65 mg/g of dry matter). The anthocyanin content of the other berries ranged from 0.65 mg/g in red raspberries to 1.55 mg/g in marionberries (4.64−11.92 mg/g of dry matter) (**Table 1**), which was similar to previous reports by Torre and Barritt, who found anthocyanin contents of 0.23−0.59 mg/g for red raspberries and 1.09 mg/g for marionberries (*25*). The relationship between ORAC and anthocyanins was not as strong as with phenolics ($r^2 = 0.932$, data not shown). This observation is in agreement with the findings of Prior and co-workers (*3*).

The anthocyanin contents are presented in **Table 2**. Using LC-MS analysis we are now able to identify various forms of anthocyanins on the basis of molecular weight and fragments

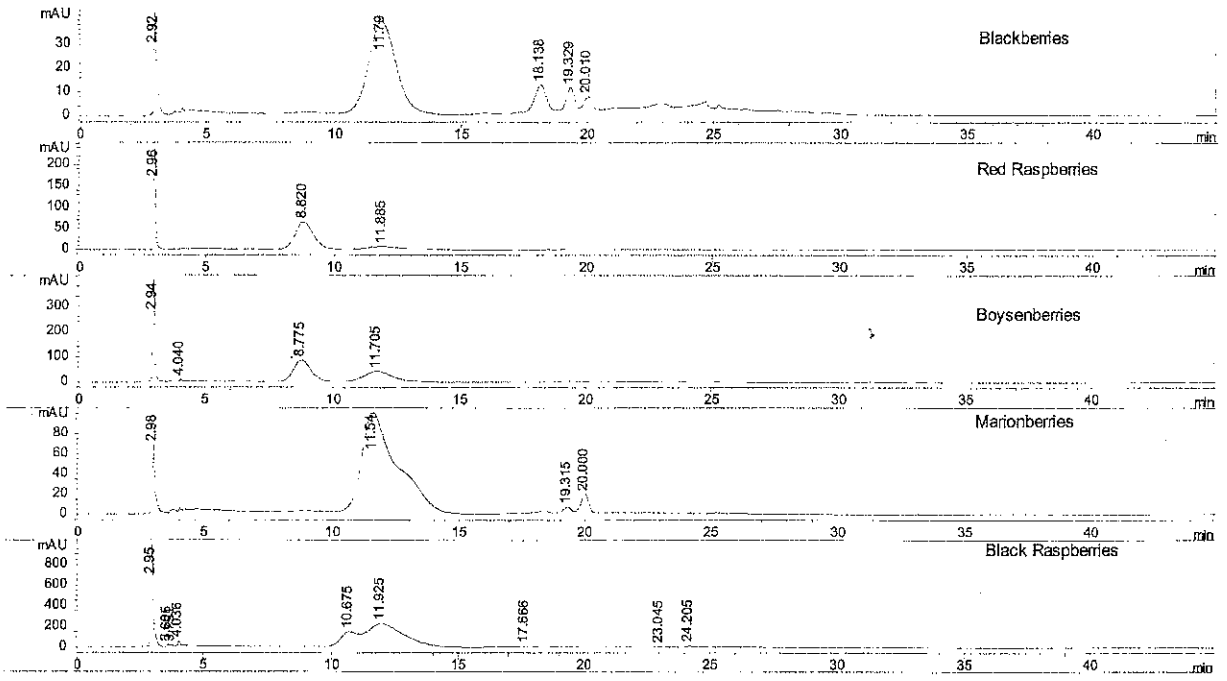

Figure 2. Chromatograms of anthocyanins from blackberries, red raspberries, boysenberries, marionberries, and black raspberries using HPLC coupled with UV detection.

Table 3. Fragmentation Pattern of Anthocyanins Present in Caneberries Analyzed by Ion Trap LC-MS

identification	retention time (min)	molecular ion $[M+1]^+$	product ion
cyanidin 3-(6′-*p*-coumaryl)glucoside-5-glucoside	8.78	757.1	611.1, 433.0, 287.3
cyanidin 3,5-diglucoside	8.82	611.0	449.2, 287.3
cyanidin 3-(6′-*p*-coumaryl)sambubioside	10.68	727.1	580.7, 287.2
cyanidin 3-(6′-*p*-coumaryl)glucoside	11.92	595.1	449.0, 287.3
cyanidin 3-glucoside	11.79	449.0	287.3
cyanidin 3-arabinoside	18.14	418.7	287.3
cyanidin 3-(6′-malonyl)glucoside	19.33	535.0	491.1, 449.0, 287.3
unknown	20.01	593.1	287.3

of product ions. The HPLC chromatograms of anthocyanin profiles are illustrated in **Figure 2**, and their mass data are shown in **Table 3**. Cyanidin was the only anthocyanidin present in all of the berries, and the *m/z* for the aglycon was 287. The anthocyanins in evergreen blackberries and marionberries were predominantly cyanidin 3-glucoside (80.4%) and cyanidin 3-(6′-*p*-coumaryl)glucoside (94.9%). For red raspberries, cyanidin 3,5-diglucoside is the major anthocyanin (89.3%). The primary anthocyanins in boysenberries were cyanidin 3-(6′-*p*-coumaryl)-glucoside-5-glucoside and cyanidin 3-glucoside (56.27 and 43.73%, respectively), whereas cyanidin 3-(6′-*p*-coumaryl)-sambubioside and cyanidin 3-(6′-*p*-coumaryl)glucoside were the primary anthocyanin forms present in black raspberries (22.0 and 77.0%, respectively). Others have identified cyanidin 3-sophoroside as a major anthocyanin in red raspberries and boysenberries (*25*–*27*) and cyanidin 3-rutinoside as a major anthocyanin in black raspberries (*25*, *28*); however, we did not find evidence for these specific compounds based on the mass data.

Although the strong association between total phenolics and ORAC may be due in large part to the presence of anthocyanins, we also chose to study other individual phenolic compounds. Among the various phenolic compounds that were measured, only gallic acid, rutin, isoquercitrin, and ellagic acid were identified and quantified in the samples. Gallic acid was present in evergreen blackberries, marionberries, and boysenberries at 0.02, 0.03, and 0.09 mg/g, respectively. Rutin was present in all berries except boysenberries, at levels of 0.11 mg/g in red raspberries and marionberries, 0.19 mg/g in black raspberries, and 0.24 mg/g in evergreen blackberries. Isoquercitrin was found only in evergreen blackberries at 0.06 mg/g (**Table 4**). In the present study, due to the lack of commercial standards, we could not completely elucidate the profile of phenolics present in caneberries. However, our primary LC-MS data reveal that many phenolic compounds are existing as glycosylated forms. To completely identify each phenolic compound, extensive multi-stage mass study is needed.

Table 4. Phenolic Compounds in Berries

species	gallic acid[a] (mg/g)	rutin[a] (mg/g)	isoquercitrin[a] (mg/g)
evergreen blackberries	0.02 (0.13)	0.24 (1.50)	0.06 (0.38)
red raspberries	nd[b]	0.11 (0.79)	nd
boysenberries	0.09 (0.75)	nd	nd
marionberries	0.03 (0.23)	0.11 (0.82)	nd
black raspberries	nd	0.19 (1.11)	nd

[a] Data in parentheses expressed per gram of dry matter. [b] nd = not detected.

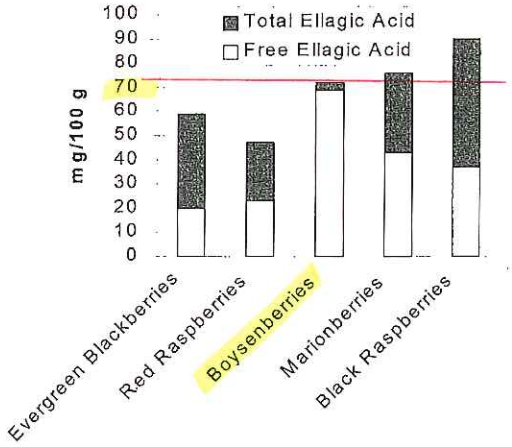

Figure 3. Total and free ellagic acid contents of evergreen blackberries, red raspberries, boysenberries, marionberries, and black raspberries.

The level of total ellagic acid ranged from 47 mg/g in red raspberries to 90 mg/g in black raspberries (**Figure 3**). The free ellagic acid level was ~40−50% of the total ellagic acid present in all berries except boysenberries. Ellagic acid was present primarily as the free form in boysenberries; thus, there was little change in concentration after hydrolysis. There has been a great deal of interest in ellagic acid as a potential anticarcinogen (*29*); however, published data on the ellagic acid content of various foods are limited. Strawberries, raspberries, and thornless blackberries (*Rubus eubatus*) have been reported to be good sources of ellagic acid (*30*−*32*). In addition to these berries, we found that black raspberries, marionberries, evergreen blackberries, and boysenberries were also rich sources.

We also found that boysenberries were unique in that essentially all of the total ellagic acid was present as free ellagic acid. Ellagic acid exists primarily as the high molecular weight form of ellagitannins in food; thus, food samples must be hydrolyzed to yield free ellagic acid so that total levels of ellagic acid can be measured (*33*). This was the situation in the other berries, where the total level of ellagic acid detected was at least twice the level measured prior to hydrolysis.

There is very little information on the absorption and metabolism of ellagitannins in man. In vitro studies with rat intestinal contents have shown that ellagitannnins can be hydrolyzed to ellagic acid at the pH found in the small intestine and cecum but not in the stomach. It is possible that the microflora of the cecum may also participate in the hydrolysis (*34*). Studies with ellagic acid rather than ellagitannins have shown that 10% of the dose given to rats was absorbed and excreted as a metabolite in urine and feces (*35*), whereas mice given higher doses showed absorption rates of ~28% (*36*). Thus, if ellagitannins must be hydrolyzed prior to absorption, boysenberries may be both high in total ellagic acid and available ellagic acid as well.

Table 5. Summary of the Oligomers from Red Raspberries

oligomer	RT (min)	molecular ion $[M-H]^-$	MW	concentration[a] (mg/g)
monomer 1	11.73	289	290	0.02 (0.14)
monomer 2	12.06	433	434	0.02 (0.14)
dimer 1	17.57	561	562	0.03 (0.21)
dimer 2	19.35	577	578	0.03 (0.21)
trimer 1	21.31	833	834	0.01 (0.07)
trimer 2	22.83	849	850	0.01 (0.07)
trimer 3	24.37	865	866	0.01 (0.07)

[a] Based on the wet weight. Data in parentheses expressed per gram of dry matter.

We were able to measure oligomeric procyanidins from monomers to hexamers, but only red raspberries had measurable levels of procyanidins. There was 0.03 mg/g of trimers, 0.06 mg/g of dimers, and 0.04 mg/g of monomers (**Figure 4** and **Table 5**). As far as we know these are the only data on the procyanidin content of caneberries. Because the whole berries were homogenized and analyzed, it is possible that these procyanidins could have been present in the raspberry seeds. Grape seeds are also a good source of procyanidins. Recently, raspberry seeds have been shown to be a good source of unsaturated fatty acids and vitamin E (*37*). Future research will look at seeds separately to see if they are also a good source of procyanidins.

Although specific levels of anthocyanins and phenolics may vary depending on factors such as ripeness and cultivar (*3*, *12*), our study found that caneberries grown in Oregon, specifically evergreen blackberries, marionberries, red raspberries, black raspberries, and boysenberries, had high antioxidant activity and

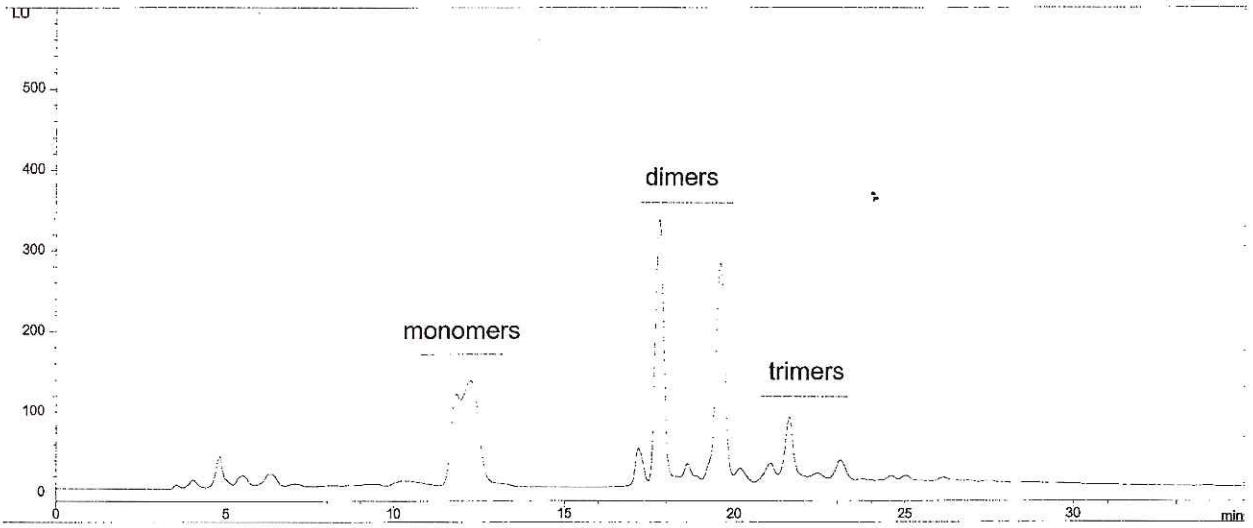

Figure 4. Procyanidin profile of red raspberries using HPLC coupled with fluorometric detection.

were rich in anthocyanins and phenolic compounds. Thus, caneberries can be an important part of a healthy diet.

ABBREVIATIONS USED

ORAC, oxygen radical absorbance capacity; Trolox, 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid; AAPH, 2,2′-azobis-(2-amidinopropane) dihydrochloride; TE, Trolox equivalent.

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