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Fatty Acid Composition and Antioxidant Properties of Cold-Pressed Marionberry, Boysenberry, Red Raspberry, and Blueberry Seed Oils

JOHN PARRY,[†] LAN SU,[†] MARLA LUTHER,[†] KEQUAN ZHOU,[†] M. PETER YURAWECZ,[‡] PAUL WHITTAKER,[‡] AND LIANGLI YU*,[†]

Department of Nutrition and Food Science, University of Maryland, College Park, Maryland 20742, and Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, College Park, Maryland 20740

Cold-pressed marionberry, boysenberry, red raspberry, and blueberry seed oils were evaluated for their fatty acid composition, carotenoid content, tocopherol profile, total phenolic content (TPC), oxidative stability index (OSI), peroxide value, and antioxidant properties. All tested seed oils contained significant levels of α-linolenic acid ranging from 19.6 to 32.4 g per 100 g of oil, along with a low ratio of $n-6/n-3$ fatty acids (1.64−3.99). The total carotenoid content ranged from 12.5 to 30.0 μmoles per kg oil. Zeaxanthin was the major carotenoid compound in all tested berry seed oils, along with β-carotene, lutein, and cryptoxanthin. Total tocopherol was 260.6−2276.9 μmoles per kg oil, including α-, γ-, and δ-tocopherols. OSI values were 20.07, 20.30, and 44.76 h for the marionberry, red raspberry, and boysenberry seed oils, respectively. The highest TPC of 2.0 mg gallic acid equivalents per gram of oil was observed in the red raspberry seed oil, while the strongest oxygen radical absorbance capacity was in boysenberry seed oil extract (77.9 μmol trolox equivalents per g oil). All tested berry seed oils directly reacted with and quenched DPPH radicals in a dose- and time-dependent manner. These data suggest that the cold-pressed berry seed oils may serve as potential dietary sources of tocopherols, carotenoids, and natural antioxidants.

KEYWORDS: α-Linolenic acid; fatty acid composition; antioxidant; phenolic; tocopherol, carotenoid; radical scavenging activity; OSI; berry seed oil

INTRODUCTION

Edible seed oils have been extracted and used as sources of food ingredients since ancient times. Today, several crops, including safflower, sunflower, corn, and soybean, are grown exclusively, or in large part, for the oil produced in their seeds. As the body of evidence that links health benefits to the consumption of vegetable oils continues to grow, many consumers now prefer to consume vegetable oils instead of animal fats. Several phytochemicals that have been detected in edible seed oils may include, but are not limited to, tocopherols, carotenoids, phenolic and polyphenolic compounds, and special fatty acids such as α-linolenic acid (18:3$n-3$). α-Linolenic acid is an essential $\omega-3/n-3$ fatty acid that cannot be synthesized in the human body and must be obtained through the diet. α-Linolenic acid may be converted to longer-chain $n-3$ fatty acids EPA (20:5$n-3$) and DHA (22:6$n-3$) *in vivo* through elongation and desaturation reactions. EPA and DHA are reported to provide potential health benefits in the risk reduction of heart disease, cancer, hypertension, and autoimmune disorders (*1−6*). Recent studies indicate that reducing the dietary ratio of $n-6$ to $n-3$ fatty acids might play a role in decreasing the risk of heart disease and cancer (*4*, *7*). The current dietary ratio of $n-6$ to $n-3$ fatty acids is about 10/1, and the recommended ratio is estimated to be 4/1 (*8*, *9*). Novel dietary sources of $n-3$ fatty acids are in high demand for improving human nutrition.

Some edible seed oils contain significant levels of other beneficial phytochemicals such as tocopherols and carotenoids. Oomah et al. found a total vitamin E equivalent of 61 mg in 100 g of cold-pressed raspberry seed oil, including α-, γ-, and δ-tocopherols at concentrations of 46.1, 144, and 7.1 mg/100 g oil, respectively (*10*). Significant levels of tocopherols were also observed in blackcurrant and goldenberry seed oils (*11*, *12*). In addition, carotenoids, steroids, and phenolic compounds are present in edible seed oils (*11*, *13*). Tocopherols and phenols are natural antioxidants that may reduce radical mediated cellular damage (*11*, *13*, *14*).

Fruit seeds are one of the major byproducts from the manufacture of fruit juice. A few studies have detected significant levels of α-linolenic acid and natural antioxidants in fruit seed oils (*10−13*, *15*). For instance, cold-pressed black

* Corresponding author. Tel: (301)-405-0761; fax: (301)-314-3313; e-mail: LY46@umail.umd.edu.
[†] University of Maryland.
[‡] Food and Drug Administration.

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raspberry seed oil contained 35% of α-linolenic acid and had significant antioxidant activities (*13*). Cranberry seed oil was a rich source of essential fatty acids, containing between 35 and 44% linoleic acid (18:2*n*−6) and 23−35% α-linolenic acid (*15*, *16*), along with significant levels of β-sitosterol and α- and γ-tocopherols (*16*). Cranberry seed oil extract showed significant radical scavenging activities against DPPH• and ABTS•+, protected protein from oxygen radical attack, and suppressed lipid peroxidation in human LDL (*17*). These data suggest that fruit seed oils might serve as potential dietary sources for natural antioxidants and other phytochemicals. Further investigation on chemical compositions and other properties of fruit seed oils is required to evaluate the potential of fruit seed oils as sources of quality oil for food applications. Cold-pressing is a seed oil extraction process that does not involve chemicals or heat prior to or during the procedure (*15*). Therefore, cold-pressed seed oil may retain more phytochemicals including natural antioxidants.

In addition to the phytochemical components, other important oil properties include oil stability, which can be measured as oxidative stability index (OSI) and peroxide value (PV). The OSI procedure determines the ability of an oil sample to withstand conditions of increased heat and airflow that accelerate its oxidative breakdown (*15*). The PV is the amount of lipid peroxides present in the oil samples. Both are indicators of overall shelf stability of edible oils.

The present study was conducted to evaluate selected commercial cold-pressed red raspberry (*Rubus ideaus*), boysenberry (*Rubus* hybrid), marionberry (*Rubus* hybrid), and blueberry (*Vaccinium corymbosum*) seed oils for their fatty acid profiles, tocopherol compositions, carotenoid profiles, total phenolic contents, oxidative stabilities, physical properties, and antioxidant activities. The information obtained from this study can be used to evaluate the potential use of these berry seed oils in food products.

MATERIALS AND METHODS

Materials. Freshly prepared cold-pressed extra virgin blueberry, boysenberry, marionberry, and red raspberry seed oils were gifts from the Badger Oil Company (Spooner WI). The berry seed oils were sampled and extracted upon arrival. 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH•), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), and gallic acid were purchased from Sigma-Aldrich (St. Louis, MO), β-cyclodextrin (RMCD) was purchased from Cyclolab R & D Ltd. (Budapest, Hungary), and 2,2′-azobis (2-amino-propane) dihydrochloride (AAPH) was obtained from Wako Chemicals USA (Richmond, VA). Wesson corn oil and Richfood soybean oil were purchased from a local supermarket. All other chemicals and solvents were of the highest commercial grade and used without further purification.

Extraction and Testing Sample Preparation. Oil constituents were extracted with both 80:20 MeOH:H_2O v/v and 100% MeOH and evaluated for DPPH• scavenging capacity, oxygen radical absorbance capacities (ORAC), and total phenolic contents. One gram of oil was measured into a test tube and then 3 mL of solvent was added. The test tube was vortexed and then centrifuged at 6000 rpm for 5 min and the supernatant was collected. This procedure was repeated two more times. All three extractions were combined and the final volume was brought to 10 mL with the extraction solvent. The resulting antioxidant solution was then kept in the dark under N_2 until further analysis.

Fatty Acid Composition. Fatty acid methyl esters (FAME) were prepared according to a previously described procedure (*18*). The FAME samples (1 μL) were subjected to both GC-FID and GC-MS analysis of fatty acid compositions. GC analysis was conducted using a Hewlett-Packard 5890 Series II gas chromatograph equipped with an FID and an HP 7673A automatic injector (Agilent Technologies, Palo Alto, CA). A fused silica CP 88 column (100 m × 0.25 mm i.d. with a 0.20-μm film thickness, Varian, Inc., Walnut Creek, CA) was used, and hydrogen was the carrier gas at a flow rate of 1.0 mL/min. Oven temperature was maintained at 75 °C for 2 min, 5 °C/min to 175 °C and held for 33 min, then 5 °C/min to 225 °C and held for 15 min. Measurements were taken in triplicate. An Agilent 6890 Network GC system equipped with Agilent 5973 mass selective detector and an Agilent 7683 automatic injector (Agilent Technologies, Palo Alto, CA) was employed for GC-MS analysis. A fused silica CP 88 column (50 m × 0.25 mm i.d. with a 0.20-μm film thickness, Varian, Inc., Walnut Creek, CA) was used with helium as the carrier gas at a flow rate of 1.2 mL/min. Initial oven temperature was 75 °C and increased to 225 °C over 80 min.

Carotenoid Composition. One milliliter of cold-pressed berry seed oil was dissolved in 160 mL of methanol/tetrahydrofuran (1:1, v/v) and analyzed for carotenoid profile using HPLC-DAD-ESI-MSMS (high performance liquid chromatography-diode-array-detector-electrospray ionization tandem mass spectrometry) method (*19*, *20*). A TSQ quantum tandem mass spectrometry (Thermo-Finnigan, San Jose, CA) was equipped with an ESI interface and Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA). HPLC separation was accomplished according to a previously described protocol with slight modification (*20*). The HPLC was performed using a Zorbax SB C18 column (Agilent Technologies, Palo Alto, CA), 50 mm × 1.0 mm i.d. with a particle size of 3.5 μm, at room temperature. The carotenoids were eluted using a mobile phase of water as solvent A and methanol: acetonitrile:2-propanol (54:44:2) as solvent B. The gradient procedure was performed as follows: (1) the gradient was linear from 50% to 99% of solvent B and the flow rate was increased from 0.2 to 0.27 mL/min in the first 10 min and (2) 99% of solvent B and flow rate of 0.27 mL/min for 10 min. The HPLC column was re-equilibrated for 10 min with 50% of solvent B, prior to the next injection. The wavelength of UV detection was set at 440 nm. The TSQ quantum was operated in the positive-ion mode under the following conditions: nitrogen (>99.7%) was used for sheath gas and auxiliary gas at pressure of 30 psi and 5 units, respectively. The temperature of the heated capillary was maintained at 300 °C, and the spray voltage of ESI was set at 4.5 kV. A collision-induced dissociation (CID) was achieved using argon as the collision gas at the pressure adjusted to more than 1.0 mTorr above the normal, and the applied collision offset energy was set to − 45 eV. Identification of carotenoids was accomplished by comparing the HPLC retention time and selected reactant monitoring (SRM) analysis of the sample peaks with that of the pure commercial carotenoid compounds (*21*). The *m/z* from 568.6 (molecular ion) to 157.3 (major fragment) was set for lutein and zeaxanthin, and *m/z* 552.6 → 145.3 and 536.6 → 119.3 were set for cryptoxanthin and β-carotene, respectively. Data were acquired with Xcalibur software system (Thermo-Finnigan, San Jose, CA). The amount of carotenoid compounds was determined using the total ion counts compared to external standards of the individual corresponding carotenoid compounds. Analyses were conducted in triplicate.

Tocopherol Profile. The methanol/tetrahydrofuran solutions of the cold-pressed berry seed oils prepared for carotenoid analysis were used to evaluate the α-, δ-, and γ- tocopherol concentrations in the oils. HPLC separation was performed using a Zorbax SB C18 column (Agilent Technologies, Palo Alto, CA), 30 mm × 1.0 mm i.d. with 3.5-μm particle size, at room temperature. The tocopherols were eluted using a mobile phase of water as solvent A and acetonitrile as solvent B. The gradient procedure was performed as follows: (1) the gradient was linear from 80% to 99% of solvent B and the flow rate was 0.3 mL/min and (2) 99% of solvent B was kept for 10 min. The HPLC column was re-equilibrated for 10 min with 50% of solvent B prior to the next injection. Identification of tocopherols was conducted by comparing the HPLC retention time and selected reactant monitoring (SRM) analysis of the sample peaks with those of the pure corresponding commercial tocopherols. The *m/z* from 430.6 (molecular ion) to 165.3 (major fragment) was set for α-tocopherol, and *m/z* 416.6 → 151.3 and 402.6 → 137.3 were set for γ-tocopherol and δ-tocopherol, respectively. The quantification for each tocopherol was accomplished using the total ion counts with external standards of the individual tocopherols, and measurements were taken in triplicate.

Total Phenolic Contents. The total phenolic contents of the selected cold-pressed berry seed oils were determined using freshly prepared Folin−Ciocalteu reagent (*22*). In brief, the reaction mixture contained 50 μL of oil extracts and 250 μL of the Folin−Ciocalteu reagent freshly prepared in our laboratory and 0.75 mL of 20% sodium carbonate and 3 mL of pure water. After 2 h of reaction at ambient temperature, the absorbance at 765 nm was measured and used to calculate the phenolic contents in oils using gallic acid as a standard. The Folin−Ciocalteu reagent was prepared by refluxing a mixture of sodium molybdate, sodium tungstate, 85% phosphoric acid, and concentrated hydrochloric acid for 10 h. This was followed by reacting with lithium sulfate and oxidizing with a few drops of bromine (*23*). The resulting solution was filtered and used for the assay. Measurements were taken in triplicate.

Oxidative Stability Index (OSI). The OSI was determined using a Rancimat instrument (Model 743; Metrohm Ltd., Herisau, Switzerland). Four milliliters of each cold-pressed berry seed oils was placed in a reaction vessel and oxidation was carried out at 80 °C with an airflow rate of 7 L/h (*15*, *22*). The OSI was defined as the hours for an oil sample to develop a measurable rancidity. The OSI values of the cold-pressed berry seed oils were compared to commercial corn and soybean oils. Triplicate measurements were conducted for each oil sample.

Peroxide Value (PV). Peroxides in the cold-pressed berry seed oils were determined using the FOX version II assay described by Nourooz-Zadeh et al. (*24*). The final FOX reagent was prepared by dissolving 880 mg BHT, 98 mg ammonium sulfate, 76 mg xylenol orange, and 100 mL of 250 mM H_2SO_4 in 900 mL HPLC grade methanol. The FOX reagent was freshly prepared and used within 24 h. The assay reaction was initiated by mixing 950 μL FOX reagent with 50 μL of oil solution or peroxide standard solution. Absorbance was determined at 560 nm following 10 min of reaction at ambient temperature. The peroxide value (PV−FOX) was calculated from a standard curve prepared using *tert*-butyl peroxide. Triplicate tests were conducted.

Determination of Refractive Index and Density. The refractive index values of the cold-pressed blueberry, red raspberry, marionberry, and boysenberry seed oils were determined at 24 °C according to the AOCS Official and Tentative Methods procedure Cc 7−25 (*25*) using an ABBE Refractometer (American Optical Corporation, Buffalo, NY). The specific density was determined at 24 °C against 4 °C pure water according to the AOCS Official and Tentative Methods procedure To 1b-64 (*26*).

Oxygen Radical Absorbance Capacity (ORAC) Assay. ORAC was evaluated using fluorescein (FL) as the fluorescent probe according to a protocol described by Huang et al. (*27*, *28*). The final assay mixture contained 0.067 μM of FL, 60 mM of AAPH, and 300 μL of oil extract or the extraction solvent for a reagent blank. The fluorescence of the assay mixtures was determined and recorded every minute. The trolox equivalent was calculated using a standard curve prepared with trolox. Measurements were conducted in triplicate

DPPH• Scavenging Activity. DPPH• scavenging capacity of the cold-pressed berry seed oil extracts was determined according to the previously reported procedure using the stable 2,2-diphenyl-1-picryhydrazyl radical (DPPH˙) (*22*). Freshly made 0.2 mM DPPH˙-MeOH solution was mixed into a cold-pressed berry seed oil extract at a concentration of 0, 6.7, 8.0, 10, 20, or 40 mg oil equivalents/mL to start the radical-antioxidant reaction. The final concentration of DPPH˙ was 100 mM and the final reaction volume was 2.0 mL. The absorbance at 517 nm was measured against a blank of pure methanol at 0.5, 1, 3, 6, 10, 20, 50, 80, and 1440 min of the reaction and was used to estimate the remaining radical levels according to a standard curve. The absorbance at 517 nm at 10 min of reaction was used to compare the DPPH• scavenging capacities of individual oil extracts. Triplicate measurements were acquired. The dose and time dependencies of cold-pressed berry seed oil extract and DPPH• reactions were demonstrated by plotting the percent of DPPH• remaining against time for each level of the seed oil extract tested.

Statistic Analysis. Data were reported as mean ± SD from triplicate determinations for each berry seed oil. Analysis of variance and least significant difference tests (SPSS for Windows, Version Rel. 10.0.5., 1999, SPSS Inc., Chicago, IL) were conducted to identify differences among means, while a Pearson Correlation test was conducted to determine the correlations among means. Statistical significance was declared at $p < 0.05$.

Table 1. Fatty Acid (FA) Compositions of the Studied Berry Seed Oils (g/100 g Oil)[a]

	red raspberry	marionberry	boysenberry	blueberry
16:0	1.3 ± 1.8	3.3 ± 0.1	4.2 ± 0.3	5.7 ± 0.4
18:0	1.0 ± 1.4	3.1 ± 0.2	4.5 ± 0.4	2.8 ± 0.1
18:1	12.4 ± 0.6	15.1 ± 0.1	18.0 ± 0.3	22.9 ± 0.1
18:2n-6	53.0 ± 1.9	62.8 ± 0.1	53.8 ± 0.3	43.5 ± 0.1
18:3n-3	32.4 ± 0.7	15.8 ± 0.1	19.5 ± 0.1	25.1 ± 0.3
sat[b]	2.3 ± 0.0	6.4 ± 0.0	8.7 ± 0.2	8.6 ± 0.3
PUFA[c]	85.5 ± 2.6	78.6 ± 0.0	73.3 ± 0.3	68.6 ± 0.3
n–6/*n*–3[d]	1.64	3.99	2.75	1.73

[a] Fatty acid composition was reported as mean ± SD ($n = 3$). [b] Sat represents total saturated fatty acid content (g/100 g oil). [c] PUFA represents total polyunsaturated fatty acids (g/100 g oil). [d] *n*–6/*n*–3 is the ratio of *n*–6 to *n*–3 fatty acids in the oil.

RESULTS

Fatty Acid Composition. Palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2*n*−6), and α-linolenic (18:3*n*−3) acids were detected in the cold-pressed red raspberry, marionberry, boysenberry, and blueberry seed oils (**Table 1**). All of the seed oils contained very high levels of total unsaturated fatty acids ranging from 91.3% (boysenberry seed oil) to 97.8% (red raspberry seed oil). Linoleic acid was the most prevalent fatty acid in all of the tested seed oils. The highest level of linoleic acid was observed in the marionberry seed oil (62.8 g per 100 g of oil). All the tested berry seed oils also contained significant concentrations of α-linolenic acid and had ratios of *n*−6 to *n*−3 fatty acids from 1.64:1 to 3.99:1 (**Table 1**). Red raspberry seed oil exhibited the lowest ratio of *n*−6/*n*−3 fatty acids, whereas the marionberry seed oil demonstrated the highest ratio among the four tested seed oils.

Carotenoid Composition. Zeaxanthin, the pigment that gives corn its characteristic yellow color, was the predominating carotenoid in all tested seed oils with a range of 5.1−13.6 mg/kg oil and comprised a combined average of over 75% of the total carotenoids (**Table 2**). Boysenberry seed oil contained the highest amount of total carotenoids and was significantly higher than all other seed oils in β-carotene, lutein, and zeaxanthin (**Table 2**). The cold-pressed red raspberry seed oil contained the highest concentration of cryptoxanthin among all tested berry seed oils.

Tocopherol Profile. The cold-pressed blueberry, red raspberry, marionberry, and boysenberry seed oils differed in their α-, γ-, δ-, and total tocopherols. The boysenberry seed oil, although lowest in α-tocopherol, showed the highest total tocopherols followed by the red raspberry seed oil (**Table 3**). The red raspberry seed oil exhibited the highest concentration of α-tocopherol (150.9 mg/kg oil). This amount was significantly higher than all other tested berry seed oils ($p < 0.0001$) and more than twice the concentration found in the next highest seed oil (**Table 3**). Boysenberry seed oil contained significantly higher concentrations of both γ- and δ-tocopherols than all others tested ($p < 0.001$) (**Table 3**).

Oxidative Stability Index (OSI). The oxidative stability index (OSI) is a measurement of the induction time of lipid peroxidation in oil or fat samples. Higher OSI values are associated with better oxidative stability or longer shelf life. OSI values of the berry seed oils were determined and compared to those of commercial corn and soybean oils (**Table 4**). It is understandable that commercial soybean and corn oils are not

Table 2. Carotenoid Contents in the Cold-Pressed Berry Seed Oils[a]

	β-carotene (μg/kg)	zeaxanthin (μg/kg)	lutein (μg/ kg)	cryptoxanthin (μg/kg)	total carotenoids (μmol/kg)
blueberry	1352.3b ± 4.4	7800c ± 600	60.6c ± 0.0	1486.8b ± 1.8	19.0
red raspberry	82.2d ± 1.8	5100d ± 300	78.6b ± 0.5	1812.9a ± 11.6	12.5
marionberry	442.7c ± 8.7	11 900b ± 800	53.3d ± 0.1	890.7c ± 14.9	23.4
boysenberry	2405.2a ± 3.3	13 600a ± 300	97.7a ± 1.0	717.6d ± 34.8	30.0

[a] Carotenoid contents were reported in mean ± SD ($n = 3$). Different letters within each column represent significance difference ($p < 0.05$).

Table 3. Tocopherol Contents in the Studied Cold-Pressed Berry Seed Oils[a]

	α-tocopherol (mg/kg)	γ-tocopherol (mg/kg)	δ-tocopherol (mg/kg)	total tocopherols (μmol/kg)
blueberry	71.1b ± 1.1	33.6d ± 0.6	6.0d ± 0.0	260.6
red raspberry	150.9a ± 1.6	558.7b ± 7.4	178.9b ± 0.4	2135.4
marionberry	28.4c ± 0.2	328.3c ± 2.2	50.0c ± 1.8	978.0
boysenberry	20.8d ± 0.1	688.6a ± 5.9	232.0a ± 2.1	2276.9

[a] Tocopherol contents were reported as mean ± SD ($n = 3$). Different letters within each column represent significance difference ($p < 0.05$).

Table 4. Peroxide Values, Oxidative Stability Index, Refractive Index, and Density of the Studied Berry Seed Oils[a]

	OSI (h)	PV (meq O–OH/kg)	refractive index	density (g/mL)
blueberry	NA	41.4a ± 2.73	1.4783	NA
red raspberry	20.30e ± 0.50	46.5ab ± 1.59	1.4788	0.929
marionberry	20.07e ± 0.35	85.2d ± 0.96	1.4774	0.934
boysenberry	44.76c ± 0.83	41.3a ± 1.41	1.4758	0.948
corn oil	65.99a ± 0.42	47.5ab ± 0.55	NA	0.932
soybean oil	46.82b ± 0.38	NA	NA	NA

[a] Data were reported in mean ± SD ($n = 3$). PV was expressed as milliequivalents of *tert*-butyl peroxide per kg of oil (meq O–OH/kg). Different letters within columns represent significance difference ($p < 0.05$, $n = 3$). NA: not available.

produced by cold-pressing and are included in this study as reference materials to reflect how OSI values may be linked to the shelf life for the berry seed oils. Boysenberry seed oil had the highest OSI value among all tested berry seed oils (44.76 h). This value is comparable but shorter than that of about 66 and 47 h observed for commercial corn and soybean oils, respectively. OSI values were 20.07 and 22.5 h for marionberry and red raspberry seed oils, respectively. The OSI values were significantly correlated to the percent of linolenic acid present in the berry seed oils ($r = -0.892$, $p = 0.017$).

FOX Peroxide Value (PV−FOX). The PV−FOX value is a determination of the total number of lipid peroxides present in the oil. A higher PV−FOX is associated with a larger concentration of existing peroxides. The tested berry seed oils exhibited a PV−FOX range of 41.3−85.2 milliequivalents of *tert*-butyl peroxide per kg oil (mequiv O−OH/kg oil) (**Table 4**). The cold-pressed marionberry seed oil showed the highest PV−FOX value among all samples, 85.2 mequiv O−OH/kg oil, whereas the boysenberry seed oil had the lowest value of 41.3 mequiv O−OH/kg (**Table 4**). The PV−FOX value of the commercial corn oil was determined to be 47.5 mequiv O−OH/kg oil, under the same experimental conditions.

Refractive Index and Density. Refractive index and density are shown in **Table 4**. The density of the commercial corn oil was also included for comparison (**Table 4**).

Total Phenolic Contents (TPC). Phenolic compounds were extracted from the berry seed oils using 80% and 100% MeOH for TPC determination. TPC values of the 100% MeOH extracts ranged from 1.27 to 2.00 mg gallic acid equivalents/g oil (mg GAE/g oil), whereas that of the 80% MeOH extracts ranged from 0.09 to 1.00 mg GAE/g oil (**Figure 1**), suggesting that the phenolic compounds in the oils may be less polar. In the corresponding berry seed oils, 100% MeOH extracts had higher TPC than the 80% MeOH extracts, suggesting the possible influence of extracting solvent on TPC estimation. Among all 100% MeOH extracts, the red raspberry seed oil showed the highest TPC, followed by that of 100% MeOH extracts of the boysenberry, blueberry, and marionberry seed oils, but 80% MeOH of the blueberry seed oil had the greatest TPC among all 80% MeOH extracts of the tested oils (**Figure 1**), indicating that these berry seed oils might differ in their phenolic compound compositions aside from their TPC.

Oxygen Radical Absorbance Capacity (ORAC). ORAC values of the analyzed seed oils are shown in **Figure 2**. The 100% MeOH extracts of the individual berry seed oils exhibited higher ORAC values than the corresponding 80% MeOH extracts (**Figure 2**), suggesting that the majority of the antioxidants present in the berry seed oils were less polar compounds and 100% MeOH is a preferred solvent for ORAC estimations of the berry seed oils. The 80% MeOH extract of the blueberry seed oil had the highest ORAC value of 32.6 μmol TE/g oil among all tested 80% MeOH extracts. The 100% MeOH extract of the boysenberry seed oil demonstrated the highest ORAC value of 77.9 μmol TE/g oil among all tested samples (**Figure 2**).

DPPH• Scavenging Activity. The berry seed oils significantly differed in their DPPH• scavenging activities (**Figure 3**). In agreement with the ORAC measurements, the 100% MeOH extracts of the individual berry seed oils exhibited stronger DPPH• scavenging activities than the corresponding 80% MeOH seed oil extracts (**Figure 3**). The highest DPPH• scavenging capacity was detected in the 100% MeOH extract of the boysenberry seed oil, followed by that of red raspberry, blueberry, and marionberry seed oils. In addition, DPPH• scavenging activity of the berry seed oils was both time- and dose-dependent. The time and dose effects of the 100% MeOH extract of the blueberry seed oil are shown in **Figure 4**A, and those of the 80% MeOH extract of the raspberry seed oil are presented in **Figure 4B**.

DISCUSSION

The present study showed that cold-pressed blueberry, red raspberry, marionberry, and boysenberry seed oils contained significant amounts of α-linolenic acid, the $n-3$ essential fatty acid for humans. The red raspberry seed oil contained 32.4% α-linolenic acid, which is comparable to that of 35.2−35.3% detected in the cold-pressed black raspberry seed oil (*13*) and greater than that of 22.3, 29.1, and 19.3% observed for cold-pressed cranberry, raspberry (*Rubus idaeus* L.), and hemp seed oils, respectively (*10*, *15*), indicating that the red raspberry seed oil is an excellent dietary source for α-linolenic acid. The

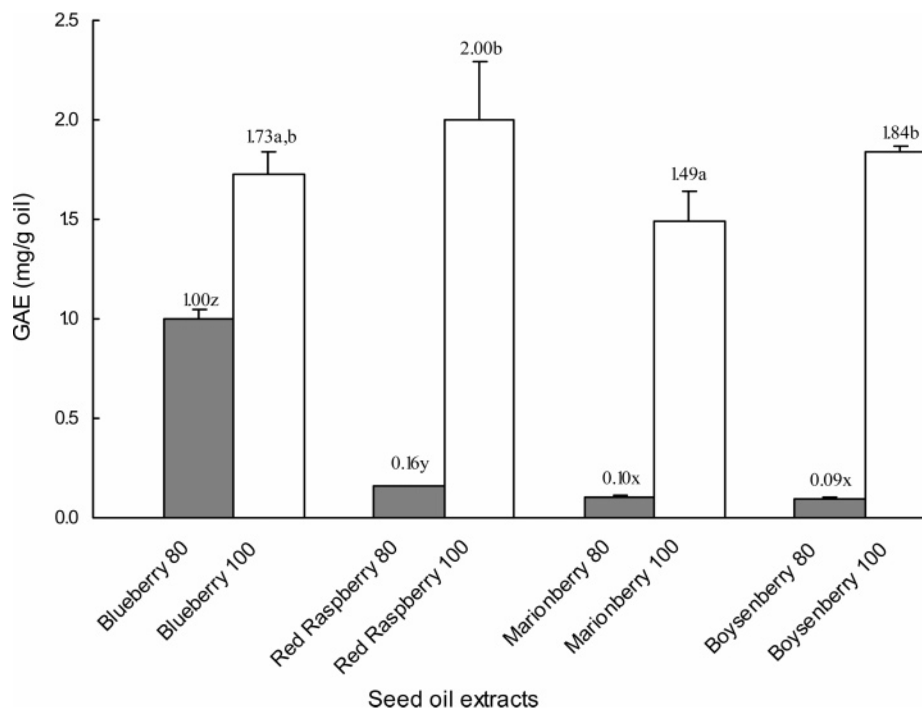

Figure 1. Total phenolic contents of the cold-pressed berry seed oils. Solid bar represents the 80% MeOH extraction, while the open bar represents the 100% MeOH extraction of each cold-pressed berry seed oil. GAE stands for the gallic acid equivalents. The vertical bars represent the standard deviation ($n = 3$) of each data point. Letters z, y, and x indicate significant differences among 80% MeOH extracts, whereas letters a and b represent significant differences among 100% MeOH extracts ($p < 0.05$).

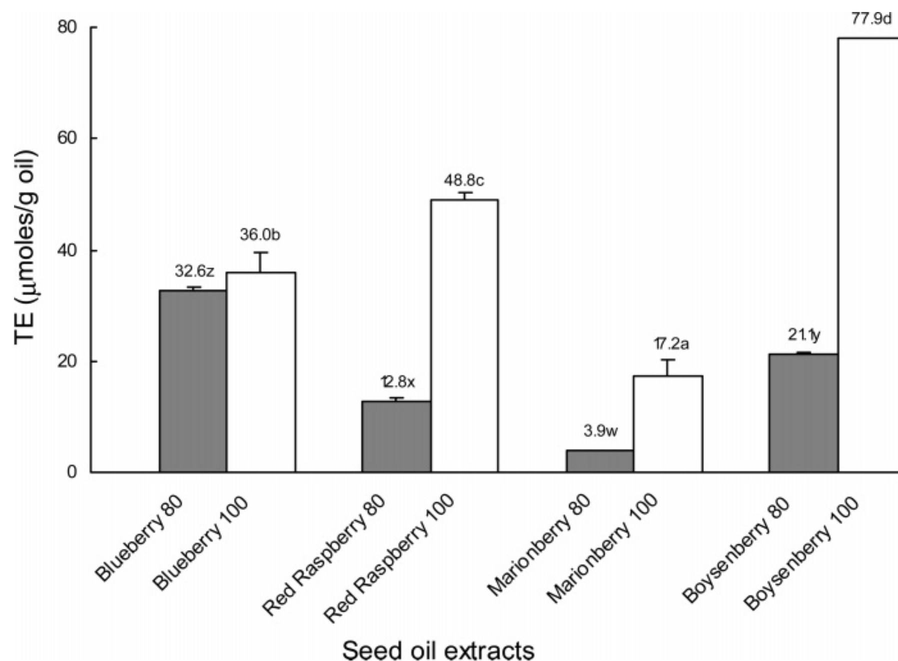

Figure 2. ORAC values of the cold-pressed berry seed oils. Solid bar represents the 80% MeOH extraction, while the open bar represents the 100% MeOH extraction of each cold-pressed berry seed oil. TE stands for the trolox equivalents. The vertical bars represent the standard deviation ($n = 3$) of each data point. Letters z, y, x, and w indicate significant differences among 80% MeOH extracts, whereas letters a, b, c, and d represent significant differences among 100% MeOH extracts ($p < 0.05$).

blueberry, marionberry, and boysenberry seed oils had α-linolenic acid contents of 15.8−25.1% and may be used as dietary sources for α-linolenic acid as well. In addition, linoleic acid (18:2n−6), the n−6 essential fatty acid, was the predominating fatty acid in the blueberry, red raspberry, marionberry, and boysenberry seed oils, ranging from 43.5% in the blueberry seed oil to 62.8% in the marionberry seed oil. Essential fatty acids cannot be synthesized in the human body and have to be obtained through the diet. Since the fruit seeds are byproducts from fruit processing, development of food applications of the berry seed oils may improve the value of these berry fruits and enhance the agricultural economy.

Similar to cranberry, hemp, and black raspberry seed oils, the blueberry, marionberry, red raspberry, and boysenberry seed oils also had very low ratios of n−6 to n−3 fatty acids (*10*, *13*, *15*), suggesting that these berry seed oils may be used to reduce

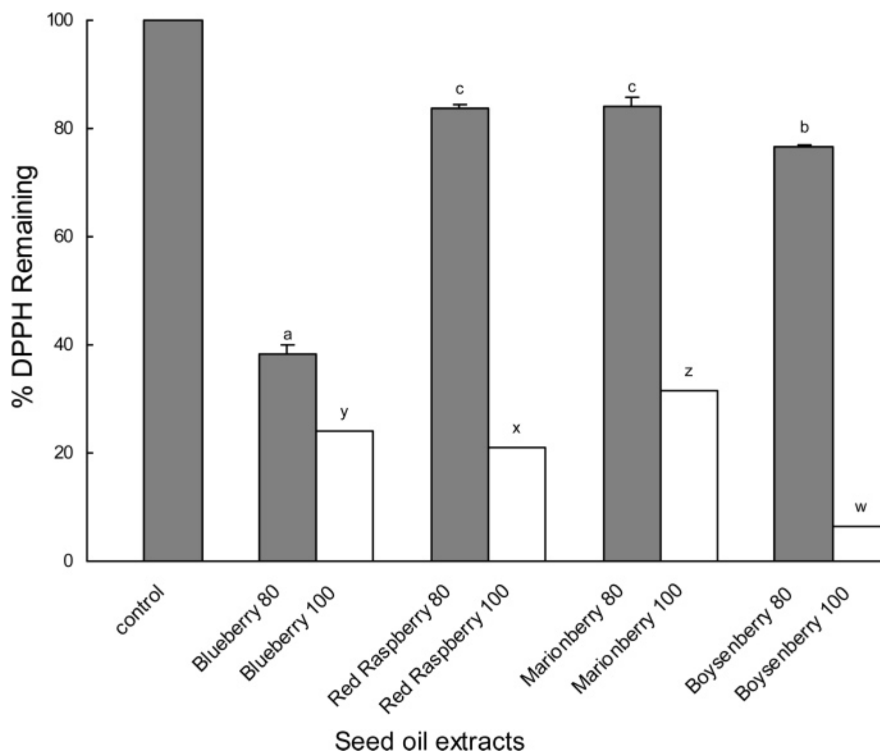

Figure 3. DPPH radical scavenging properties of the cold-pressed berry seed oils. Solid bars represent the 80% MeOH extracts of each cold-pressed berry seed oil, while the open bar represents the 100% MeOH extracts of each oil. The initial DPPH• concentration was 100 μM in all reaction mixtures, while the final concentration of the berry seed oil extracts was 40 mg oil equivalent per mL. The vertical bars represent the standard deviation ($n = 3$) of each data point. Bars with different letters are significantly different $p < 0.05$. Letters a, b, and c indicate significant differences among 80% MeOH extracts, whereas letters z, y, x, and w represent significant differences among 100% MeOH extracts ($p < 0.05$).

the ratio of $n-6$ to $n-3$ fatty acids in the diet. Growing evidence suggests that the reduction in the $n-6/n-3$ ratio may be beneficial in reducing the risk of the development of cancer, enhancing bone health, and reducing the risk of cardiovascular disease (*4*, *29*−*32*).

Significant concentrations of tocopherols and carotenoids were detected in the cold-pressed berry seed oils. The boysenberry seed oil, although lowest in α-tocopherol, had the highest total tocopherols followed by the red raspberry seed oil. In the present study, the boysenberry seed oil contained 20.8, 688.6, and 232.0 mg α-, γ-, and δ-tocopherols per kg of oil, respectively, which is very different from the previous reported concentrations of 880, 9080, and 8440 mg/kg lipids, respectively, for α-, γ-, and δ-tocopherols by Ramadan and Morsel (*11*). Ramadan and Morsel (*11*) also reported a level of β-tocopherol at 11.3 g/kg lipids (11300 mg/kg lipids), which makes the total tocopherol concentration of about 2.9−3.0% in total lipids. The difference between the two studies cannot be explained by the different growing conditions. In agreement with the observation by Oomah et al. (*10*), the major tocopherol in the red raspberry seed oil from the current study was the γ isomer and accounted for about 63% of total tocopherols. Oomah et al. (*10*) detected α-, γ-, and δ-tocopherols in both cold-pressed and hexane-extracted red raspberry seed oils, and γ-tocopherol was 75% of the total tocopherol. Interestingly, Oomah et al. reported a ratio of α- and δ-tocopherols at 6.5 to 1 or 4.1 to 1 for cold-pressed and hexane-extracted red raspberry seed oils, respectively, but was 0.83 to 1 in the red raspberry seed oil from the present study. This difference may reflect the variation among raspberry genotype and the influence of growing conditions on phytochemical production. Genotype, growing condition, and the interaction between genotype and environmental conditions may alter the phytochemical composition in botanicals (*22*). All of the cold-pressed berry seed oils contained lower amounts of α-tocopherol than tested commercial extra virgin olive, peanut, corn, and sunflower seed oils with a range of 174−578 mg/L. However, the values from the present study were similar to that of 89 mg/L in the soybean oil (*33*). Among the berry seed oils tested in the present study, boysenberry seed oil contained the highest amount of total carotenoids and demonstrated the greatest β-carotene content of 2405 μg/kg oil or 2280 μg/L oil. This amount was much higher than that detected in peanut (130 μg/L), soybean (280 μg/L), and corn (1200 μg/L) oils investigated by Cabrini et al. (*33*).

Among all 100% MeOH extracts, the boysenberry seed oil extract exhibited strongest scavenging activity against both DPPH• and peroxyl radicals induced by AAPH followed by that of red raspberry, blueberry, and marionberry seed oil extracts, respectively. For the 80% MeOH extracts, the blueberry seed oil extract had the highest radical scavenging capacity against both tested radicals, while the boysenberry extract had the lowest radical scavenging capacity. This may be because individual solvent systems had different extraction efficiencies for different antioxidant compounds. The effects of extraction solvent on antioxidant activity estimation were also observed and discussed in detail in our previous study of wheat antioxidants (*34*).

In conclusion, the present study indicated that the cold-pressed red raspberry, marionberry, boysenberry, and blueberry seed oils might serve as excellent dietary sources for α-linolenic acid, essential fatty acids, tocopherols, and carotenoids. This study also showed that the tested berry seed oils contain significant levels of natural antioxidants. Utilization of these berry seed oils in food and cosmetic products may enhance the profitability of fruit production and processing industries.

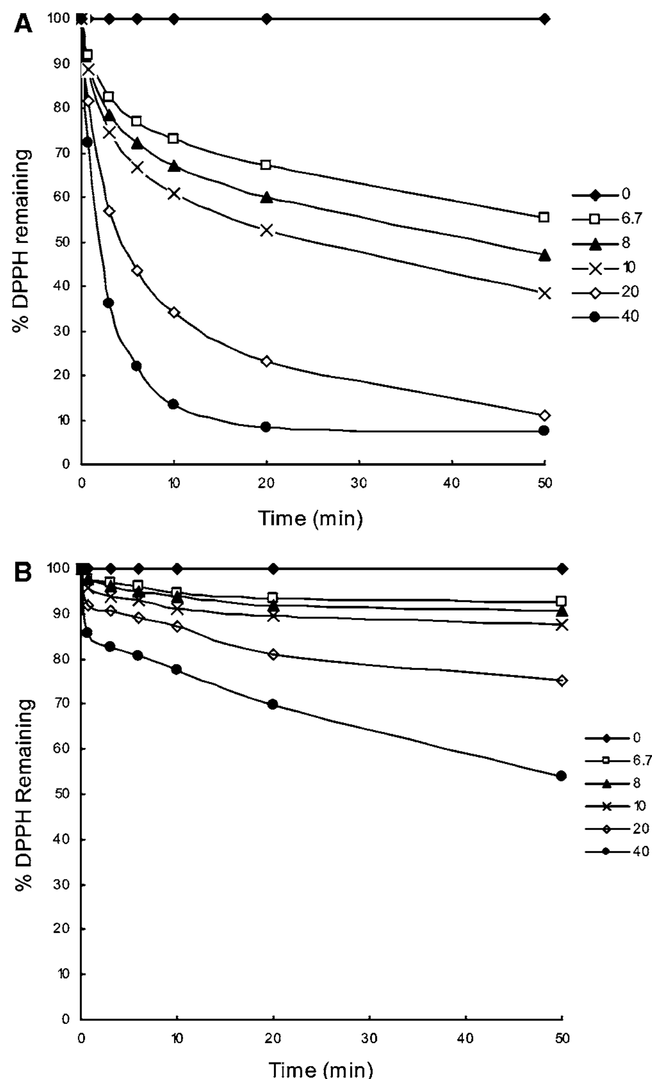

Figure 4. Dose and time effects of the oil antioxidants–DPPH• reactions. (**A**) represents the dose and time effects of 100% MeOH extracts of the blueberry seed oil and DPPH• reactions, whereas (**B**) represents the dose and time effects of 80% MeOH extracts of the raspberry seed oil and DPPH• reactions. 0, 6.7, 8, 10, 20, and 40 represent the final concentrations of the seed oil extracts at 0, 6.7, 8, 10, 20, and 40 mg oil equivalents per mL in the antioxidant–radical reaction mixtures, respectively. The initial DPPH• radical concentration was 100 μM in all reaction mixtures.

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